

**Phosphite in Soil and Turfgrass**

by

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## Abstract

Phosphate, derived from phosphoric acid ( $\text{H}_3\text{PO}_4$ ), has long been used as a P fertilizer source, while phosphite, derived from phosphorous acid ( $\text{H}_3\text{PO}_3$ ), has long been used as a known and proven fungicide. However, the exact role of phosphite is unclear – is it functioning as a fertilizer, fungicide, or both? Previous work in crop production has shown that phosphite can be detrimental to plant growth, especially if the soil is low in phosphate. Two greenhouse studies (repeated in time) and one incubation study were used to evaluate the behavior of phosphite in the soil and its effects on ryegrass and bentgrass growth. Phosphite-containing materials (both labeled fungicides and phosphite-containing fertilizers) were applied based on P rate or at labeled rates. Collected data included root and shoot growth, and P uptake. In early sampling periods (1 month) application of P as phosphite did negatively affect root and shoot growth. However, by two months after fertilization phosphite had likely converted to phosphate, and plant growth was either unaffected or improved. Fungicide-only treatments indicated that this was a function of P and not fungicidal activity of phosphite. Results from the incubation study support this conclusion as phosphite was converted to phosphate largely within the second sampling month. Further work should investigate if similar results are observed in the field, and at what point in time effects of phosphite are mitigated by conversion to phosphate.

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## List of Abbreviations

TSP	Triple super phosphate
IC	Ion chromatography
ICP	Inductively coupled plasma spectrometry

## Literature Review

### Phosphorus in the soil

Phosphorus (P) is the second primary element (next to nitrogen) limiting agricultural production in most regions of the world. Found only in a few minerals, the ultimate source of all soil P is primary apatites (Smeck, 1985). Apatite is a group of phosphate minerals,  $\text{Ca}_5(\text{PO}_4)_3(\text{F},\text{OH},\text{Cl})$ , usually containing fluorapatite, hydroxylapatite, and chlorapatite, with high concentrations of  $\text{F}^-$ ,  $\text{OH}^-$ , and  $\text{Cl}^-$  ions, respectively, in crystal formation (Hughes and Rakovan, 2002). All apatite minerals contain phosphate oxyanions linked by  $\text{Ca}^{2+}$  cations. The main P-bearing mineral in igneous rocks is an early-formed accessory mineral, fluorapatite (Filippelli, 2008). Phosphorus is associated with authigenic carbonate-fluorapatite in sedimentary rocks. The global biogeochemical cycling of phosphorus starts by its release from apatite at the surface of earth, and finally develops to the formation of other geological apatites through sedimentary processes or tectonic recycling (Hughes and Rakovan, 2002). In soils, weathering releases P from apatite minerals by several processes. Microbial respiration releases  $\text{CO}_2$  resulting in increased acidity around degrading organic matter and root hairs (Schlesinger, 1997). Poorly crystalline P-bearing minerals dissolve rapidly in this acidic condition, releasing P to root pores. Also, plant roots exude organic acid, which can dissolve apatite and release P to soil pore spaces (Schlesinger, 1997).

The largest amount of P in soil is grouped as organic P, which is unavailable for plants in that form (Holford, 1997). Organic P is found primarily as ester linkages on inositols, with lesser amounts in phospholipids and nucleic acids (Cosgrove, 1977). Even though organic P is unavailable for plants, the incorporation of inorganic fertilizer P into the soil organic P pool, and organic P mineralization emphasize the importance of organic P in soil P cycling. Labile organic

P contributed 83 to 93% of that mineralized (Sharpley, 1995). It has been estimated that rates of P mineralization from organic P to inorganic P amounted to about 15 to 33 kg P ha<sup>-1</sup> yr<sup>-1</sup> (Sharpley, 1995) and 16 to 23 kg P ha<sup>-1</sup> yr<sup>-1</sup> (Reddy, 1983), respectively.

Plant available P exists only as inorganic P ions from the soil solution. Orthophosphate ions, which are important inorganic ligands in soil solution, are easily adsorbed by plants. In most soils, H<sub>2</sub>PO<sub>4</sub><sup>-</sup> and HPO<sub>4</sub><sup>2-</sup> are the major orthophosphate ions (Hinsinger, 2001). Phosphate ions are derived from dissociation of orthophosphate, and are very strongly adsorbed to colloids, especially with Fe and Al hydrous oxides, because of their extremely high surface area and their overall negative charge (Holford, 1997).

Phosphorus is extremely reactive and is only available for plant uptake within a narrow range of neutral soil pH values. In acid soil, trivalent Fe and Al (Fe<sup>+3</sup> and Al<sup>+3</sup>) are the dominant cations to combine with phosphate. Conversely, Ca<sup>+2</sup> forms ion pairs with phosphorus in neutral or alkaline soil (Hinsinger, 2001). Inorganic phosphorus easily precipitates with metal cations, forming mineral P. The relative content of these insoluble phosphorus forms in soil are very high, but nearly unavailable for plant use. In comparison, weakly adsorbed P (often called 'labile' P) can readily be desorbed back into the soil solution (Holford, 1997).

### **Phosphite**

Phosphite is a generic name used to describe alkali metal salts of phosphorus acid (H<sub>3</sub>PO<sub>3</sub>). The other name for phosphite is phosphonate, which is more commonly used to describe products made of the salts or esters of phosphorous acid (Varadarajan et al., 2002). The most common phosphite in fertilizer or fungicide is potassium phosphite. Potassium phosphite is also referred to as mono (KH<sub>2</sub>PO<sub>3</sub>) and di-potassium (K<sub>2</sub>HPO<sub>3</sub>) salts of phosphorous acid on some phosphonate product labels (Thao and Yamakawa, 2009).

Plants take up phosphite ions, but they are not used in phosphorus metabolism, whereas phosphate is readily accessed and utilized by the plant (Schachtman et al., 1998). Although phosphite is not the ideal form of P for plants, it is an important part of the global P cycle due to its oxidation to phosphate by microbes (Varadarajan et al., 2002). Previous studies have found that phosphite is a poor source for nutritional P, and cannot be used directly by plants, because the conversion of phosphite (Phi) to phosphate (Pi) in soil is too slow for plant use (MacIntire et al., 1950). A similar result was observed with *Saccharomyces cerevisiae* (yeast), which was unable to use phosphite P as a P source (McDonald et al., 2001b).

The approximate half-life of phosphite oxidation to phosphate in soil has been shown to be 12-16 weeks. In that study soil microorganisms (such as *Pseudomonas fluorescens*) metabolized phosphite to phosphate (Adams and Conrad, 1953). Due to this slow conversion, some plants grew better in the second year after applying phosphite fertilizer (McDonald et al., 2001a). In other work, soil phosphate content increased slightly after 7 days to (74 mg kg<sup>-1</sup>) as compared to that measured in control plots (52 mg kg<sup>-1</sup>) after applying 7.5 g P m<sup>-2</sup> as KH<sub>2</sub>PO<sub>3</sub> (potassium phosphite) solution (1905 mg P). After 30 days, extractable PO<sub>4</sub><sup>3-</sup> had increased to 199 mg kg<sup>-1</sup>, with highest phosphate concentration (330 mg kg<sup>-1</sup>) detected two months after applying phosphite to the soil (Stöven et al., 2007). One of the reasons for the slow conversion of phosphite to phosphate is that the microbial community prefers using phosphate as the P source, rather than phosphite (Lovatt and Mikkelsen, 2006). Others found that not until there was insufficient phosphate in the culture did microbes use phosphite as the resource for oxidation (Adams and Conrad, 1953). Phosphite metabolism initially requires the absorption and assimilation of phosphite by some soil dwelling bacteria, such as *Escherichia coli*, *Pseudomonas stutzeri*, *Alcaligenes faecalis* and *Xanthobacter flavus* (White and Metcalf, 2007). Phosphite is

then enzymatically oxidized to phosphate before being transferred into organic forms (Adams and Conrad, 1953). There are two important purposes for the oxidation of phosphite for these microbial communities: the production of energy and the production of phosphate. Phosphite may be toxic to crops in the first cropping cycle. This is most likely to occur when soil phosphate is low (Thao and Yamakawa, 2009).

### **Phosphate versus Phosphite**

Phosphite and phosphate are derived from phosphorous acid ( $\text{H}_3\text{PO}_3$ ), and phosphoric acid ( $\text{H}_3\text{PO}_4$ ), respectively. Phosphorous acid contains only two ionizable hydrogen atoms, as compared to phosphoric acid, which has three ionizable hydrogen atoms (Young, 2004). In solution, phosphorous acid dissociates to  $\text{H}_2\text{PO}_3^-$  and  $\text{HPO}_3^{2-}$ . However, phosphoric acid ( $\text{H}_3\text{PO}_4$ ) can form  $\text{PO}_4^{3-}$  easily, while  $\text{PO}_3^{3-}$  can't be formed in solution (Lovatt, 1990). The difference is that one hydrogen atom in the phosphorous acid binds with the phosphorus atom through a covalent bond, and thus does not react with neutralizing agents. All three hydrogen atoms in phosphoric acid bind with the phosphorus atom through ionic bonds. As a result, the acid strength of phosphorous acid is close to five times stronger than phosphoric acid, equal to that of sulfuric acid (McDonald et al., 2001a). When phosphorous acid ( $\text{H}_3\text{PO}_3$ ) is neutralized with a base such as sodium hydroxide (NaOH), potassium hydroxide (KOH), or ammonium hydroxide ( $\text{NH}_4\text{OH}$ ), salts of phosphorous acid will form (Lovatt, 1990).

Phosphate is considered to be the only phosphorus source for plant nutrition. Thus, phosphates are recommended for plant fertilization (Moor et al., 2009). Phosphorus fertilizers, such as triple super phosphate (0-46-0), ammonium phosphate (18-46-0 or 11-52-0), and potassium phosphate, readily disassociate to produce plant available P, hydrogen phosphate ( $\text{HPO}_4^{2-}$ ) and dihydrogen phosphate ( $\text{H}_2\text{PO}_4^-$ ) (Thao and Yamakawa, 2009). Phosphites and

phosphates are both mobile in both xylem and phloem (Moor et al., 2009).

Phosphate concentrations in soil may affect phosphite toxicity. Cerioni et al. (2013) found that increased phosphate decreased phosphite inhibition of *Penicillium digitatum*. In their study, using Pratt's medium at pH 6, the phosphite concentration that inhibited germination of 50% of conidia ( $EC_{50}$ ) was 469 mg L<sup>-1</sup> with excess phosphate (10mM). In comparison, radial growth of *Rhizopus stolonifer*, *Fusarium oxysporum*, and *Verticillium dahlia* was inhibited at a much lower phosphite concentration of 69 mg L<sup>-1</sup>, when a low phosphate concentration was included (0.084mM) (Cerioni et al., 2013). A similar result was found by Fenn and Coffey (Fenn and Coffey, 1984). Their research showed that a phosphite concentration of 552 mg L<sup>-1</sup> reduced radial growth rate of *Alternaria alternata* and *Rhizoctonia solani* by 59 and 38%, respectively, in a cornmeal agar which contained high concentration of phosphate at pH 6.2. When the phosphate content was increased, toxicity of phosphite to these fungi decreased significantly (Fenn and Coffey, 1984).

In Brazil, maize (*Zea mays* L) was fertilized with phosphite and phosphate at four different P combinations. Phosphorus was supplied as either 100% phosphate or 75% phosphate and 25% phosphite at P rates of 52 $\mu$ M (low) and 644 $\mu$ M (adequate). Results indicated that the phosphite supply affected phosphorus nutrition and biochemical responses in maize plants, and it was found that phosphite could not substitute for phosphate as a P source. Root and shoot dry mass weight, and total leaf blade area per plant were reduced significantly in treatments in which 25% of the P was phosphite. Results of this work also showed that phosphite only affected biomass production of maize when the plants were grown in low P soils (52 $\mu$ M) (Ávila et al., 2011).

Other researchers found similar results. When tomatoes were grown with technical or commercial grade phosphite, foliar symptoms due to phosphorus deficiency were observed,

including darker green leaves and reddish or purplish spots on the lower leaf. Seven different P solutions were evaluated in this work. These were a zero P solution, 0.1 mM phosphate solution, 1 mM phosphate solution, 0.1 mM phosphite solution, 1 mM phosphite solution, 1mM phosphate/0.3 mM phosphite solution, and a 1mM commercial phosphite solution. All tomatoes grown with phosphate showed better growth compared to the no-phosphorus control, or plants only fertilized with phosphite. Total leaf areas and dry weights of leaves, roots, and stems of tomatoes fertilized with only phosphate as the P source were approximately 2 to 5 times greater than those fertilized with only phosphite and no-phosphorus control treatments (Förster et al., 1998). Combinations of 1mM phosphate and 0.3 mM phosphite were also found to increase tomato leaf area, when compared with applying phosphate and phosphite alone (Förster et al., 1998).

### **Phosphite as a fungicide.**

Phosphite is used primarily as a fungicide, instead as a source of P nutrition. Reacted with ethanol, phosphite forms ethyl-phosphonate, which is considered as a conventional postharvest fungicide. Ethyl-phosphonate is widely applied as a fungicide under the trade-name Aliette® (Bayer CropScience LP, Research Triangle Park, North Carolina, 27709) or Fosetyl-Al (McDonald et al., 2001a).

Some fungi in *Oomycetes*, such as *Phytophthora ciricola* and *Phytophthora cinnamomi* are inhibited by phosphite. In addition, phosphite may play a role in the maturation of fruits, disease resistance, yield, and other quality aspects of agroindustrial products (Dametto et al., 2007). The function site of phosphite is not in the host plant, but within the fungal pathogen (Fenn and Coffey, 1984). Detached tomato leaves and grape leaves, inoculated with *P. capsici*, or *Plasmopara viticola*, produced more antifungal compounds, such as phenolic compounds,

antifungal stilbenes and flavonoids, when treated with fosetyl-A1 (Cohen and Coffey, 1986).

Phosphites enhanced disease resistance in potato (*Solanum tuberosum* L.) through induced resistance (IR), which is defined as the mechanism that, upon abiotic or biotic stimuli, plants increase their level of resistance against a future stress (Machinandiarena et al., 2012). Potassium phosphite (Afital Potassium Phosphite, Agro-EMCODI SA) was applied to foliage at 10 mL per plant (3 L/ha), and disease symptoms caused by *Phytophthora infestans*, a causative agent of potato late blight, were reduced (Machinandiarena et al., 2012). Other work indicated that phosphite was an excellent fungicide for control of downy mildew of soybean (*Glycine max* L.) (Silva et al., 2011). The area under the Disease Progress Curve (AUDPC) of downy mildew decreased linearly with increasing phosphite rate from 0 to 1500 g a.i. ha<sup>-1</sup>. At the highest rate of phosphite application disease was reduced by 50% (Silva et al., 2011). The incidence of *Peronospora manshurica* on seed was reduced linearly by increased phosphite at rates from 0 to 1500 g a.i. ha<sup>-1</sup>. Highest rates of phosphite application reduced pathogen incidence on seed by 83% (Silva et al., 2011).

Another study evaluated crown rot of pepper (*Capsicum annuum* L.) as affected by phosphite (Förster et al., 1998). Crown rot caused by *P. capsici* significantly decreased in pepper plants to which phosphite had been applied rates of 0.1 mM or 1 mM, compared to the control treatment and phosphate treated pepper. Two weeks after inoculation, plants in the control treatment began to die, and as the study continued plants in the phosphate treatments began to develop disease. In comparison, phosphite treated plants were still healthy after four weeks (Förster et al., 1998).

Phosphite has also been shown to have good efficacy against *Penicillium expansum*, which can cause blue mold disease in pome fruit (Amiri and Bompeix, 2011). This work also showed that phosphite was more effective when heated (50°C). The authors suggested that conversion of



$K_2HPO_3$  and  $KH_2PO_3$  to  $H_3PO_3$  was faster with increasing temperature. Rebollar-Alviter et al. (2010) found that potassium phosphite (AgriFos, 4.6 L/ha, 45.8% mono- and di-potassium salts of phosphorous acid) provided excellent control of leather rot of strawberry caused by *Phytophthora cactorum* (Rebollar-Alviter et al., 2010).

Sudden oak death (SOD) is a forest disease caused by the pathogen *Phytophthora ramorum*. The study of bark application of phosphite, or phosphite injection for controlling SOD in coast live oak showed that phosphite injections were effective in decreasing the growth of *Phytophthora ramorum* in inoculated potted coast live oak trees (Garbelotto et al., 2007). Soil drenches and topical bark applications of phosphite produced no significant reduction in disease, and foliar applications resulted in inconsistent lesion reduction. The beneficial effect of foliar treatments were lost after only 8 weeks. However, preventive injections and comprehensive bark application of phosphite mixed with the organosilicate Pentrabark™ were the most useful and consistent treatment for controlling sudden oak death, and the effect of injection lasted for 8 months after application (Garbelotto et al., 2007). In all treatments, oaks treated with phosphite had smaller lesion diameters than in untreated oaks, a result attributed to phosphite slowing the growth of the pathogen. Bock et al. (2012) reported that phosphite fungicide (potassium phosphite, 54.5% a.i., at the standard rate of 2.64L ProPhyt 1000L<sup>-1</sup> ha<sup>-1</sup>, Helena Chemical Company, Collierville, TN) was advantageous for controlling pecan scab caused by *Fusicladium effusum*, improving fruit quality early in the season (Bock et al., 2012).

When combined with other fungicides or fertilizers, the addition of phosphite often improves plant performance. The combination of ammonium phosphate and ammonium phosphite was more effective for providing phosphorus to the plant than either ammonium phosphate, diammonium phosphite or potassium phosphite alone (Young, 2004), but the mechanism was not

clear. Generally, in a heated solution, the fungicide performance of phosphite was improved when added to other labeled fungicides (Cerioni et al., 2013). The combination of potassium phosphite (KPhi) and imazalil (IMZ), a commercial fungicide, was more effective than either IMZ or KPhi alone for preventing Eureka lemon or Valencia orange from developing green mold after inoculation with *Penicillium digitatum* (Cerioni et al., 2013). The addition of KPhi increased IMZ effectiveness when the treatment solutions were held at 50°C, as compared to those held at 25°C. This is perhaps because the higher temperature increased degradation of  $K_2HPO_3$  and  $KH_2PO_3$  to their active form ( $H_3PO_3$ ), and enhanced fungus uptake of potassium (Amiri and Bompeix, 2011). This combination is common in the canning industry, and used to effectively control IMZ-resistant isolates of *P. digitatum*. Cerioni et al. (2013) also found that pH affected the inhibitory activity of phosphite on conidial germination. The phosphite inhibitory activity for germination of conidia of *P. digitatum* was greater at a lower pH (pH 3), twice as toxic at pH 7. The phosphite concentration that inhibited germination of 50% of the conidia was 229 mg L<sup>-1</sup> at pH 3 and 498 mg L<sup>-1</sup> at pH 7 (Cerioni et al., 2013).

With a long term use of phosphite in disease control, the chemical mutagenesis of some fungi does occur. For example, although *Phytophthora* species are suppressed under application of phosphite, resistance is evolved after several years of continuous application. A report showed that two phosphite-resistant *Phytophthora* have been obtained by chemical mutagenesis (McDonald et al., 2001a).

In turfgrass, studies have been conducted to evaluate the effect of phosphite as a fungicide. Phosphonate fungicide has been shown to inhibit *Pythium spp.* and suppression of pythium blight on turfgrass (Cook et al., 2009). Phosphonate treatments, potassium phosphite, potassium phosphite-C, Fosetyl-Al, Fosetyl-Al/pigment, and Mefenoxam were compared with potassium

phosphate and a control. Results showed that all phosphonate treatments suppressed blighted turf on perennial ryegrass and creeping bentgrass, as compared to the control and potassium phosphate treatment. When phosphorous acid was applied at the same rate of phosphite as in potassium phosphite and fosetyl-Al fungicides, the effect of suppression on Pythium blight was similar. Several phosphite fungicides (Chipco Signature, Alude) were tested for dollar spot (*Sclerotinia homoeocarpa*) control on a creeping bentgrass putting green. Effects were similar, and all provided good control of dollar spot, with some improved turf quality compared to untreated controls. Although the active ingredient of these fungicides were all phosphite, observed differences in the improvement in turfgrass quality were likely due to differences in formulation (Vincelli et al., 2005).

#### **Direct and indirect mechanisms of disease control with phosphite**

Oomycetes are a class of filamentous eukaryotic pathogens that infect and colonize plant tissue through secreting effectors (Bozkurt et al., 2012). Phosphites have been found to be effective on diseases (late blight on potatoes, blue mold on tobacco, grape downy mildews, etc.) caused by oomycetes fungi of the order peronosporales such as *Pythium* spp, *Phytophthora* spp, *Peronospora* spp and *Plasmopora* spp (Silva et al., 2011; Cohen and Coffey, 1986). They have also been shown to be ineffective if oomycetes are not present. Two major mechanisms are involved in disease reduction by phosphites. First is the direct inhibition of mycelia growth (Smillie et al., 1989). For example, applications of phosphite to lupin inhibited growth of mycelia of *Phytophthora cinnamomi*, but the rate of lesion development was not affected (Smillie et al., 1989). It was thought that phosphite (applied at 20 mg of phosphite per pot) was acting directly on the fungi, because lupin lacked an active dynamic defense system to ward off the disease.

The second mechanism by which phosphite works is an indirect effect, stimulating plant defense mechanisms, increasing the amount of phytoalexins and reactive oxygen species (ROS). This strengthens the induction of pathogenesis-related proteins (PRs), which are peroxidases related to the induction of plant defense mechanisms (Lobato et al., 2011), as reflected by intensified guaiacol-peroxidase activity. This defense mechanism plays a critical role in suppressing pathogen growth, increasing cell walls, and enhancing lignin biosynthesis of leaves (Ávila et al., 2011). The application of phosphite affected plant structures, as the periderm and cortex of potato tubers had highest pectin content in cell walls of potassium phosphite (KPHi) treated tubers. Also, proteinase inhibitors, which are important in plant defense mechanisms, were increased by applying KPHi in both periderm and cortex tissues (Olivieri et al., 2012).

Phosphite fertilization of strawberries (*Fragaria ananassa*) was not better than phosphate fertilization for increasing yields, but application of phosphite stimulated plant defense mechanisms, such as increasing the formation of bioactive compounds, and enhancing total antioxidant activity of strawberry (Moor et al., 2009). In this study, Phosfik, a widely used phosphite fertilizer in Europe, was applied to strawberries, and it was compared to phosphate fertilized strawberries. Fruit weight was not influenced by phosphite fertilizer treatments, and total yield of strawberries were not increased. However, some bioactive compounds in plant, such as ascorbic acid and anthocyanin, were increased with phosphite application, which suggests an activation of plant defense mechanisms. Soaking plants in phosphite fertilizer solution before planting was favorable both for ascorbic acid and anthocyanin formation (Moor et al., 2009).

Sometimes both mechanisms are involved, following the application of phosphite. For example, when phosphite was applied to tobacco at a rate of 20 mg per pot, invasion by *P.*

*nicotianae* was decreased, and lesion extension was eliminated at 48 hours after application (Smillie et al., 1989). Protection lasted over 5 weeks, and the concentration of phosphite in stem tissue didn't decrease throughout the entire experimental period. The researcher demonstrated both direct and indirect mechanisms of disease suppression were involved. Phosphite not only provided protection against invasion by fungi, but it also stimulated a complex dynamic system, with the capacity to synthesize phytoalexins. Other work indicated that the mechanism that dominates (direct or indirect) for disease control depends on the phosphite concentration applied (Carswell et al., 1997). When a low concentration of phosphite was applied to the soil, that phosphite stimulated the host defense enzymes at the site of pathogen ingress in the roots. When a higher concentration of phosphite was applied, it not only stimulated the host defense enzyme, but it also directly inhibited pathogen growth. Phosphite can also be used for scab control in some plant species, such as apple (*Malus domestica*) and pecan (*Carya illinoensis*) (Bock et al., 2012).

### **Phosphite as a fertilizer**

The possibility of registration of phosphite as a P fertilizer is affected by the definition that the composition of a P fertilizer should be expressed in terms of P<sub>2</sub>O<sub>5</sub> (Moor et al., 2009). Phosphite requires oxidation to be utilized by the plant, and competes with phosphate for transport sites, and this process may take months, so it is not an effective source of phosphorus for plants (Silva et al., 2011). For example, in one study, phosphite applications did not increase content of potassium or phosphorus in the leaf tissue of soybean. In the second year of the work, there was a significant reduction in tissue P when phosphite was continuously applied (Silva et al., 2011). Yield of soybean in the first growth season was 920 kg ha<sup>-1</sup> higher than in the second growth season, and no interaction was observed between phosphite rate and yield. No interaction was

observed between phosphite rate and sources for seed weight either.

The effect of phosphite as a P source is highly dependent on the phosphate status of the plants (Thao et al., 2008b). In most studies, the use of phosphite as a fertilizer source shows that it is not effective. Most report that phosphite had deleterious effects on growth and metabolism of the plants, resulting in a P deficiency (Thao et al., 2009). For example, Carswell et al., 1997 found that relatively low phosphite concentrations (1-2 mM) did not affect phosphate-fertilized oilseed rape (*Brassica. Napus* L.) suspension cells, but phosphate-starved oilseed rape was far more susceptible to the deleterious effects of phosphite (Carswell et al., 1997). The work also demonstrated that intracellular phosphate levels in *Brassica spp.* were reduced by 50 to 60% seven days after treatment with phosphite in phosphate-deprived plants, while the concentration of phosphite in leaves and roots of phosphate-fertilized plants was significantly lower than that of phosphate-deprived plants. They also found that the presence of phosphite caused a 20 to 25% reduction in whole-seedling fresh weights, but only in the phosphate-starved seedlings. This is because some plant proteins (APase, PFP) that recognize phosphite as phosphate suppress the phosphate-starvation response of plant under a sufficient phosphite level.

In a study with hydroponically cultivated celery, phosphite applied at 0.1 to 2 mmol L<sup>-1</sup> into both low (0.05 mmol L<sup>-1</sup>) and high (0.5 mmol L<sup>-1</sup>) phosphate supplied solution did not improve plant growth (Thao et al., 2009). When phosphite was applied at 2 mmol L<sup>-1</sup>, the growth of low phosphate-fertilized celery was significantly decreased. In other phosphite versus phosphate research with different phosphate:phosphite ratios, the same total amount of P was applied to spinach (*Spinacia oleracea* L.) roots at either low or high total P levels. Results indicated that no matter what the level of total P, plant growth was significantly reduced as the proportion of phosphite increased (Thao et al., 2008a). Other work by the same researchers found that lettuce

supplied with phosphate for approximately 80% of its maximum growth was harmed when combined with a low phosphite concentration of 0.2 mmol L<sup>-1</sup>. However, when the lettuce was fertilized with phosphate to obtain 90% of maximum growth, the plant was only harmed by phosphite at a high rate (2 mmol L<sup>-1</sup>). Thus, if phosphate is limiting, less phosphite can cause damage. Additionally, even additional phosphite applications, reaching to 2 mmol L<sup>-1</sup>, did not affect lettuce yield if sufficient phosphate (0.3 mmol L<sup>-1</sup>) was present (Thao and Yamakawa, 2009).

Increased root: shoot ratios are a hallmark of plants deficient in phosphate, especially in spinach and tomato (Thao and Yamakawa, 2009). When combinations of phosphite and phosphate were evaluated on komatsuna (a common leafy vegetable in Japan), it was found that when the ratio of phosphate:phosphite was 3:1 or 1:0, shoot dry weight of komatsuna was unaffected, whereas shoot dry weight was reduced when the phosphate:phosphite ratio was decreased to 1:1 and 1:3 (Thao et al., 2008a). The application of 5 mM phosphite reduced root: shoot fresh weight ratio and whole-seedling fresh weights of phosphate-starved oilseed rape (*Brassica. Napus* L.) (Carswell et al., 1997). The same result was found with leguminous plants. Foliar application of phosphite decreased shoot and grain dry weight of bean (*Phaseolus vulgaris* L.) when soil phosphate was deficient, and there was no effect when the beans were grown in high phosphate soil (Ávila et al., 2012). Regardless of the level of soil P, the addition of phosphite reduced the shoot and grain dry weight of bean, when compared to treatments to which no phosphite had been added (Ávila et al., 2012).

The reason behind the ability of phosphite to further harm plants grown in phosphate deficient soils was explored in work by Varadarajan et al. (2002). This study explored the effect of phosphite and phosphate application on phosphate starvation-induced gene expression in

tomatoes (Varadarajan et al., 2002). Application of phosphite first inhibited phosphate starvation-induced gene expression, and then caused visible morphological changes in plants, such as accumulation of anthocyanin in leaves and stem. The researchers believed that the cause of suppression of phosphate starvation was the intervention of phosphite in signal transduction, with the pathway considering phosphite as phosphate. As a result, plants could not perceive phosphate deficiency, even in an extremely low concentration of phosphate (Varadarajan et al., 2002). Phosphate starvation inducible genes, such as LePT2 (high affinity Pi transporters), LePS2 (APase) and TPSI1 (novel genes) were not expressed in phosphate-absent tomato plants when phosphite was present in the growth media (McDonald et al., 2001a). In a study with *Brassica. spp.*, the enzyme (APase) and transporters (high-affinity plasmalemma phosphate translocator) of the phosphate starvation response were reduced by application of phosphite, with a 75% reduction in APase (McDonald et al., 2001b).

Another possible detrimental effect of phosphite is that the continuous application of phosphite may affect soil microflora (McDonald et al., 2001b). A large amount of phosphite in soil could influence microorganism populations. For example, the roots of plants that form symbiotic associations with beneficial fungi would be changed with significant amounts of phosphite in the soil. Microorganisms which have ability to utilize phosphite as a P source would be selected over those that utilize phosphate (McDonald et al., 2001a).

Although there are proven negative effects of phosphite on plant growth, some researchers still believe that phosphite has a greater role than just serving as a fungicide, if used in an appropriate way (Moor et al., 2009; Smillie et al., 1989). Phosphite fertilizers have been recommended as foliar fertilizers in consideration of environmental pollution and food safety, as they have extremely low toxicity to invertebrates, animals (including humans) or aquatic



organisms in natural ecosystems (Moor et al., 2009). In a study of phosphite-treated plants (lupin, tobacco), the highest concentration of the P anion was found in the stem, and lowest found in roots, so the phosphite ion appears to have an advantage when applied in a solution form to plant foliage (Smillie et al., 1989). Foliar application of potassium phosphite increased yield of Satsuma orange (*Citrus unshiu* L.) through stimulating flowers and fruit setting, compared to the control and phosphate treatment (Thao and Yamakawa, 2009). These results were not observed with all plant species.

Because there are many commercial phosphite products in the turfgrass market, the objective of this research was to: 1) examine commercial phosphite fertilizers for their effect on ryegrass, 2) evaluate the time of conversion of phosphite to phosphate in soil, and 3) determine if fungicide rates of commercial phosphite products negatively affected bentgrass growth.

## Materials and Methods

Three different experiments were conducted for this research project. Two greenhouse studies (both repeated in time) evaluated commercial phosphite materials sold either as fertilizer or fungicide. A laboratory incubation study (conducted once) evaluated the conversion of phosphite to phosphate.

### Greenhouse study I – ryegrass

Two runs of this study were conducted over the period from 24 January 2014 to 22 September 2015 at the Plant Science Research Center (Auburn, AL). The first experiment was conducted from 18 April 2014 to 10 June 2014 (7 weeks). It was conducted using pots (14.5 cm diameter) containing 100% sand into which perennial ryegrass was seeded. For this study, 1.8 g (0.11 kg m<sup>-2</sup>) ‘Eagle Select’ perennial ryegrass seed blend (cultivars were Playoff II, Allsport 3, and Greenville) was placed on top of 1365 g sand, and another 70 g of sand covered the seed. The second study period was 12 August 2015 to 22 September 2015 (6 weeks). For this experiment a Marvyn loamy sand (fine-loamy, kaolinitic, thermic Typic Kanhapludult) was used, and the experiment was set up as described previously. Sand and soil were collected from Auburn University Turfgrass Research Unit (Auburn, AL). Before the start of the experiment, bulk soil samples were analyzed for nutrient content. Soil pH was 7.4, and no nutrient deficiencies were detected (Table 1).

This experiment evaluated three P sources: Turfite (Headland Amenity Ltd., Caldecote, Cambridge), TKO (Growth Products, White Plains, NY), and triple super phosphate (Table 2). These three P sources were all applied at four P rates: 13, 26, 40 and 52 kg P ha<sup>-1</sup>. All materials were converted from phosphite- and phosphate-P content to a P basis for equal observation. Three fungicide treatments were also included: Alude (Cleary Chemical Corp., Dayton, NJ),

Headway (Syngenta Crop Protection LLC., Greensboro, Carolina), and Chipco Signature (Bayer Environmental Science, Research Triangle PK, NC), all applied at labeled rates (Table 2). Fungicides were added treatments because of the fungicidal nature of phosphite. In an attempt to separate fungicide from fertilizer effects both phosphite containing (Alude, Chipco Signature) and non-phosphite containing (Headway) fungicides were included. Five replications were used for each treatment.

Phosphorus treatments were incorporated with soil once at the beginning of the study before seeding, and all other nutrients were supplied weekly via a P-depleted Hoagland's nutrient solution, applied at 20 mL per pot to supply (4.2 mg N pot<sup>-1</sup>) per week. Fungicide treatments were applied as shown in Table 2. Pots were watered as needed to prevent turfgrass stress. All pots were placed randomly on the greenhouse bench every day.

Clippings were harvested every month from each pot in the first run, with 2 harvests total, and were harvested once at the end of the study in the second run. Dry weight of clippings was recorded and clippings were saved for tissue P analyses, following standard procedures (AAES, 1986), followed by analysis via inductively coupled plasma spectrometry (AAES, 1986). Dry weight of harvested roots was determined in Run 2 of the study. Phosphorus uptake was determined by multiplying tissue dry weight by phosphorus concentration.

### **Greenhouse study II – bentgrass**

This study was conducted using creeping bentgrass that was established as vegetative samples (7 cm diameter) in 14 cm (height) by 14.5 cm (diameter) pots. Bentgrass ('Penn G-2') was collected from a 5 year old putting green at Auburn University Turfgrass Research Unit (Auburn, AL) on 27 July 2015. Plugs were washed clean of soil, and were placed into pots filled with a USGA-type greens mix (Hummel, 1993). Pots were filled with 500 g of a USGA-type mix (80%

sand, 20% peat by volume). Soil test analyses were performed on this mix (Table 1). All experiments were conducted in the greenhouse (Plant Science Research Center, Auburn, AL).

This study consisted of 5 P rates (0, 15, 30, 60, 120 kg P ha<sup>-1</sup>), with and without phosphite sources that were applied at labeled fungicidal or product rates (Table 3). Phosphite products were Alude (a phosphite fungicide), and Title Phyte (phosphite fertilizer). Following labeled directions, Alude was sprayed every other week and Title Phyte was applied weekly, at labeled rates. These rates were 296 mL Alude per 93 m<sup>2</sup> and 118 mL Title Phyte per 93 m<sup>2</sup> (approximately 12.9 kg P ha<sup>-1</sup> as phosphorous acid in each Alude treatment, 7.3 kg P ha<sup>-1</sup> as phosphorous acid in each Title Phyte treatment). All other nutrients were supplied weekly via a P-depleted Hoaglands solution, applied at 20 mL per pot (4.2 mg N) per week. Pots were watered every day to prevent turfgrass stress.

This study consisted of two separate runs. The first study period was 29 July 2015 to 22 September 2015 (8 weeks), and the second period was 1 October to 23 November 2015 (8 weeks). Five replications were used for each treatment. Clippings of bentgrass were collected once at the end of each study, with dry weight of clippings recorded and saved for tissue P analyses (ICP) (AAES, 1986). Roots were saved only for dry weight measurement. Phosphorus uptake was also determined by multiplying tissue dry weight by phosphorus concentration.

### **Incubation study**

This study was initiated on 2 July 2014, and continued until 15 January 2015. The study was conducted using sealable plastic tubs (17×15×13 cm) to which treatments had been added. One soil type was used: Marvyn loamy sand (Fine-loamy, kaolinitic, thermic Typic Kanhapludult). Initial nutrient analyses are shown in Table 1. Three P sources (Triple super phosphate (0-44-0), TKO, and Title Phyte) were all applied at four P rates: 56, 84, 112, and 140 kg P ha<sup>-1</sup> (Table 4).

All phosphite and phosphate in the products was converted to a P-basis for uniformity.

Each tub was filled with 500 grams of soil, and watered uniformly to 80% of field capacity. All materials were applied and mixed thoroughly with the soil. Each tub was sealed and all placed into a growth chamber at 21°C. Each week tubs were removed, opened to stimulate air movement, and resealed. Tubs were replaced randomly in the growth chamber.

After 20 days of incubation, soil samples were taken every month, with soil in each tub mixed thoroughly before sampling. During each month the following was done: 1) 2 gram subsample was removed and dried to measure soil moisture content, 2) 5 gram subsample was extracted with CaCl<sub>2</sub> (50 mL 0.01 M), and 3) 5 gram subsample was extracted with Mehlich I soil extract (Mehlich, 1953). Samples were analyzed for P via two methods. First was the determination of P via inductively coupled argon plasma spectrometry (ICP) (Morton et al., 2005). Second was the separate measurement of phosphite and phosphate via ion chromatography (IC) (Morton et al., 2005).

### **Statistical Analysis**

Because P rate was embedded as a part of greenhouse ryegrass study that included various fungicide treatments, the study was not appropriate for analysis of variance. Instead, the data was analyzed via individual treatments, with means separation to determine differences. To better analyze the effect of P rate and P source some analyses were performed with fungicide treatments removed.

Because fungicide treatments were not included as separate treatments, greenhouse bentgrass trials were appropriately analyzed using analysis of variance, with P rate and phosphite product as main effects.

## Results and Discussion

### Greenhouse Ryegrass Study

#### Dry weight of plant tissue

For the first harvest of Run 1, there were no significant differences in dry weight of perennial ryegrass due to P source, P rate or fungicide, when analyzed over all treatments (Fig 1). However, when fungicide treatments were removed from the analysis (Table 5) some differences due to treatment were apparent.

In the first harvest of Run 1 there was a linear increase in dry weight of perennial ryegrass as P rate increased, but only in plants fertilized with triple superphosphate (TSP) (Table 5). In comparison, plants fertilized with Turfite or TKO often had decreases in dry weight as P rate increased (Table 5). The two products that produced reduced ryegrass at higher rates of P both contained phosphite. A previous study has also reported a similar result that phosphite had deleterious effects on growth and metabolism of plants (Carswell et al., 1997).

In the second harvest of Run 1 differences due to P rate and source were also apparent (Fig 2). In general, perennial ryegrass treated with higher rates of P (as Turfite and TKO, especially) had greater yield than that measured in control and non-phosphite fungicide treatments (Headway). The following treatments had significantly greater dry matter yield than that measured in control plots: Turfite at 40 and 52 kg P ha<sup>-1</sup>, and TKO at rate of 52 kg P ha<sup>-1</sup> (Fig 2). Differences in yield between the two Harvests in Run 1 is likely a function of phosphite to phosphate conversion. This second harvest was performed at 8 weeks after planting, and by this time toxic phosphite would have been likely converted to phosphate (Adams and Conard, 1953). This is also supported by another previous study, which found phosphate two months after applying phosphite to soil (Stöven et al., 2007).

Application of any fungicide (Alude, Headway, and Chipco Signature) did not significantly increase ryegrass tissue dry weight, when compared to the zero P control (Figs 1, 2, 3), even when some of the fungicides were phosphite-based (Alude and Chipco Signature). Alude has an effective control of *Pythium* and damping-off diseases of turf grasses. Chipco Signature controls summer decline and anthracnose, while Headway controls anthracnose, bentgrass dead spot and brown patch. In our greenhouse trials, no evidence of disease was observed.

For all treatments, tissue dry weight in Run 2 was typically higher than that measured in Run 1. A possible reason is that background soil fertility in the Marvyn soil was higher than in sand (Table 1). Run 2 of the first experiment had few differences in plant dry weight due to P rate, P source or fungicide (Fig 3). The only significant effects were observed in perennial ryegrass treated with Turfite, where dry weight of perennial ryegrass was higher when fertilized with that source (at 26, 40 or 52 kg P ha<sup>-1</sup>), as compared to any other treatments (Fig 3). When analyzed within P source, dry weight of ryegrass increased as P rate increased (Table 5). Negative effects of phosphite were not observed, likely a function of the 8 week period until harvest.

### **P concentrations in Plant Tissue**

For all P sources, adding any level of P increased tissue P when compared to control and fungicide treatments (Figs 4, 5) in both harvests of Run 1, and in the single harvest of Run 2 (Fig 6). When P sources were analyzed separately for the effect of P rate, in every case P content in leaf tissue increased as P rate increased (Table 6).

Phosphorus content in fungicide-only treated plots was lower due to the low rate of applied P in those products, a function of using the labeled application rate (the highest applied P rate was in Alude treatment, which contained approximately 12.9 kg P ha<sup>-1</sup> phosphorous acid). It must be noted here that measured tissue P is not only for phosphate, as tissue P also includes phosphite.

Plants take up both phosphite and phosphate, although phosphite ions are not used in phosphorus metabolism (Schachtman et al., 1998). Phosphite and phosphate are both mobile in xylem and phloem and both would be measured in tissue P (Moor et al., 2009).

Uptake of P (Harvest 1, Run 1) was affected by P source and P rate, but not at low rates of fertilization (12 kg P ha<sup>-1</sup> for all P sources, and 26 kg P ha<sup>-1</sup> for Turfite) (Fig 7). By the second harvest of Run 1 almost every treatment in which P was applied resulted in increased tissue P, when compared to the control (Fig 8). The only exceptions were the treatments of TKO and TSP applied at the lowest P rate (13 kg P ha<sup>-1</sup>), where tissue P was not significantly greater than that measured in the control. As with P concentrations, P uptake would reflect both phosphite and phosphate.

Phosphorus uptake in Run 2 typically increased as P rate increased (Fig 9). This occurred with all P sources. In most cases P uptake was greater when P was added, with the exception of the lowest P rate applied as TKO and TSP. This is the same result as observed in Run 1 (Fig 8).

### **Dry weight of Ryegrass Roots**

When analyzed by individual treatments, the dry weight of perennial ryegrass roots was unaffected by P rate, P source or fungicide (Fig 10). Weight of roots as affected by P rate (when analyzed within P source) indicated a curvilinear response to increasing P (Fig 11). In all cases root dry weight was maximized at a P rate at or near 40 kg P ha<sup>-1</sup>. The only exception to this was roots fertilized with Turfite, where the dry weight of roots was greatest at a P rate of 26 kg P ha<sup>-1</sup> (Fig 11). Turfite was a phosphite-containing material.

Previous work has showed that phosphite can be detrimental to plant growth in crop production (Förster et al., 1998; Silva et al., 2011). In our work, early harvests often showed similar results (Table 5), while later harvests (after 8 weeks) often showed no ill effects to



applications of phosphite. These later results likely occurred because plant material was not harvested until after 8 weeks of growth. Phosphite may have converted to phosphate in that time, providing adequate P for plant use. Sampling more frequently may help to determine if any early-period effects due to phosphite can be found.

### **Greenhouse Bentgrass Study**

The intent of the second greenhouse study was to explore the effect of labeled rates of phosphite products on bentgrass growth, when applied in combination with rates of phosphate fertilizer. Since this experiment was a standard P rate by P source study, analysis of variance was used to separate treatment effects.

Dry weight of bentgrass tissue was unaffected by P rate, nor was the interaction significant (Table 7). However, analysis of variance for bentgrass dry weight showed that the effect of phosphite source was significant (Table 7). Application of phosphite materials at labeled rates never decreased bentgrass tissue yield, and in some cases yield was increased (Figs 12, 13). This was observed most frequently in treatments to which Alude had been applied. In Run 1, when Alude was applied to bentgrass grown in the 15, 60 and 120 kg P ha<sup>-1</sup> treatments, bentgrass tissue dry weight was significantly increased (Fig 12). In Run 2, the application of Alude increased bentgrass tissue dry weight at every soil phosphate level, except for the highest rate (Fig 13). The effect of Title Phyte was not significant. No negative effect was found, even when soil was low in phosphate. Alude was a labeled fungicide, and phosphite concentration in Alude as applied at labeled application rate was higher than that in Title Phyte (approximately 12.9 kg P ha<sup>-1</sup> phosphorous acid in each Alude treatment, and 7.3 kg P ha<sup>-1</sup> phosphorous acid in each Title Phyte treatment), so the increased bentgrass tissue yield for Alude was likely due to its fungicidal effect.

An interaction of P rate and P source on P content was found in both Runs (Table 7). This was because when no phosphite was added, P content typically increased as P rate increased, and this was not observed when Title Phyte and Alude were applied (Table 8). Application of materials with phosphite increased tissue P (Table 8). In both Runs, bentgrass sprayed with Alude had greater tissue P than that sprayed with Title Phyte, or that receiving no phosphite, when bentgrass was grown in the 0 and 15 kg P ha<sup>-1</sup> treatments (Figs 14, 15).

Uptake of P was significantly affected by the interaction of P rate and P source in Run 1 (Table 7), but not in Run 2. In Run 1, this was because the uptake response at 30 kg P ha<sup>-1</sup> was unaffected by the application of phosphite, while in all other cases P uptake increased with phosphite treatment (Fig 16). In Run 2, P uptake increase when any product with phosphite was added (Fig 17).

There was also a significant interaction for the effect of P rate and phosphite product on bentgrass root dry weight (Table 7). In most cases, application of phosphite products decreased bentgrass root dry weight when P rate was low (Figs 18, 19). This negative effect was mitigated when P fertilization rates were higher, and there were no differences due to treatment when P was applied at 120 kg P ha<sup>-1</sup>. A greater root dry weight was observed in the Alude and Title Phyte treatments at the highest P rate (120 kg P ha<sup>-1</sup>). Previous studies have reported a similar result, that root growth of phosphate deficient komatsuna (*Brassica rape* var. *peruviridis*) was sensitive to phosphite (Thao et al., 2008b). There was an obvious decrease in root growth with decreasing phosphate: phosphite ratios, and severe root damage was found when phosphate: phosphite ratio was 0:100.

## **Incubation Study**

Two extraction methods were used:  $\text{CaCl}_2$  and Mehlich-1 extracts. Results of  $\text{CaCl}_2$  extraction revealed low amounts of extractable P (Table 9), which were not great enough for separating total P to phosphite and phosphate on IC, so Mehlich-1 extracts were used for subsequent IC analyses.

For TKO, the conversion of phosphite to phosphate was largely completed at the second sampling time (Fig 20). In the first sampling period (20 day after initiation) virtually no P was detected as phosphate (Fig 20). At that sampling date most P was measured as phosphite, with increasing phosphite measured as P rate increased. By the second month of sampling almost all P was measured as phosphate, with no P measured as phosphite (Fig 20).

Similar results were measured for the Title Phyte product (Fig 21). In the first sampling period, acid extractable soil phosphorus as phosphite was detected, with increasing phosphite measured as P rate increased. Little measured phosphate was measured in the June sampling. One month later, measured phosphite in every Title Phyte treatment had decreased, and phosphate content increased linearly from the lowest P rate to the highest. Results from the rest of the sampling time showed that phosphite continued to change to phosphate, with greater conversion in the first 3 months (Jun-August) (Fig 21).

The inclusion of a phosphate treatment clearly shows the lack of conversion when phosphite is not a part of the soil system (Fig 22). In that case extractable P increased as P rate increased, and there was a slight increase over time as well.

In general, the majority of phosphite was converted to phosphate within 60 days in this incubation study. This is faster than results from previous studies, which found a half-life of 3 to 4 months (Adams and Conard, 1953). Based on their theory, the oxidation of phosphite to

phosphate in soil was largely due to the microbial activity within soil. An assumption can be made that soil used in this study (Maryvn loamy sand) was different from the soil used in previous studies, so different soil dwelling bacteria may have a particular microbial activity thus affects the time of oxidation of phosphite to phosphate.

## Conclusions

The greenhouse ryegrass study demonstrated early period toxic effects of phosphite products on plant growth, within the rates used in this study. The effect was mitigated after 8 weeks, likely a result of phosphite conversion to phosphate. Application of phosphite materials at labeled rates to bentgrass did not negatively affect dry weight of plant tissue, and in some cases it was increased. However, when soil P was low (low rates of added P) bentgrass root growth was decreased. This effect disappeared when P was added at rates greater than 60 kg P ha<sup>-1</sup>.

In the incubation study, phosphite was converted to phosphate largely within 60 days in Marvyn loamy sand, which was faster than results from previous studies. Future studies should: 1) include more frequent sampling in time to determine when differences might occur, 2) include different soil types to better understand the phosphite to phosphate conversion, and 3) test soil for P so that the soil-test P levels at which sensitivity to phosphite products may occur is known.

Table 1. Initial soil-test results<sup>†</sup> for all sand and soil used in greenhouse and laboratory experiments.

greenhouse-ryegrass									
Run 1					Run 2				
P	K	Ca	Mg	pH	P	K	Ca	Mg	pH
		mg kg <sup>-1</sup>					mg kg <sup>-1</sup>		
0	2.5	28	2	7.4	19	42	70.5	18.5	5.7
greenhouse- bentgrass									
Run 1					Run 2				
P	K	Ca	Mg	pH	P	K	Ca	Mg	pH
		mg kg <sup>-1</sup>					mg kg <sup>-1</sup>		
2	9	73.5	23.5	4.9	2	10.5	110.5	31.5	4.7
laboratory incubation									
Marvyn loamy sand									
P	K		Ca		Mg		pH		
		mg kg <sup>-1</sup>							
2	36.5		464		35.5		6.5		

<sup>†</sup>Mehlich-I soil test extraction.

Table 2. Nutrient/ active ingredient content of tested products for greenhouse perennial ryegrass study.

Commercial Trade Name	Manufacturer	Active ingredient	Phosphite Source	Phosphate Source	%N-P-K or Concentration	Application Rate
Turfite <sup>†</sup>	Headland Amenity Ltd. Cambourne, Cambridgeshire, UK	Ammonium Hydroxide Phosphoric Acid	Phosphorous Acid	None	7-8-0	NA
TKO <sup>†</sup>	Growth Products. White Plains, NY 10603	Mono- and Di-potassium Salts of Phosphorous Acid	Phosphorous acid	None	6.875 lbs of phosphite per gallon	NA
Triple super phosphate <sup>†</sup>	Piedmont Fertilizer Company. Opelika, Al 36801	Triple superphosphate	None	Triple super phosphate	0-20-0	NA
Alude <sup>‡</sup>	Cleary Chemical Corp. Dayton, NJ 08810	Mono- and Di-potassium Salts of Phosphorous Acid	Phosphorous Acid	None	3.35 lbs phosphorous acid/gallon	3.1 mL in 100 mL water. Sprayed 1.53 mL per pot
Headway <sup>‡</sup>	Syngenta. Greensboro, North Carolina 27419	Azoxystrobin Propiconazole	None	None	0.87 lb ai propiconazole and 0.52 lb ai azoxystrobin per gallon	1.21 mL in 100 mL water. Sprayed 1.5 mL per pot
Chipco Signature <sup>‡</sup>	Bayer Environmental Science. Research Triangle PK, NC 27709	Aluminum tris (O-ethyl phosphonate) Other ingredients	O-ethyl phosphonate	None	80% Aluminum tris 20% other ingredients	1.55 mL in 100 mL water. Sprayed 1.51 mL per pot

<sup>†</sup> Marked as a fertilizer, no EPA label as a fungicide

<sup>‡</sup> Fungicide

Table 3. Nutrient/ active ingredient content of tested products for greenhouse bentgrass study.

Name	Manufacturer	Active ingredient	Phosphite Source	Phosphate Source	Labeled Rate
Alude	Cleary Chemical Corp. Dayton, NJ 08810	Mono- and Di-potassium Salts of Phosphorous Acid	Phosphorous acid	None	Apply 296 mL of product in a liter of water per 93 m <sup>2</sup> . Once another week (12.9 kg P ha <sup>-1</sup> phosphorous acid)
Title Phyte	Harrell's. Lakeland, FL 33802	Soluble potash (K <sub>2</sub> O)	Phosphorous acid	None	Apply 118 mL of product in a liter of water per 93 m <sup>2</sup> . Once a week (7.3 kg P ha <sup>-1</sup> phosphorous acid)



Table 4. P-containing materials used for soil incubation test.

Trade name/ product	Phosphite	N-P <sub>2</sub> O <sub>5</sub> -K <sub>2</sub> O	Manufacturer
Triple superphosphate (TSP)	None	0-46-0	Piedmont Fertilizer Company. Opelika, AL 36801
TKO	0.34 kg P <sub>3</sub> O <sub>5</sub> L <sup>-1</sup>	0-0-26	Growth Products. White Plains, NY 10603
Title Phyte	0.29 kg P <sub>3</sub> O <sub>5</sub> L <sup>-1</sup>	0-0-30	Harrell's. Lakeland, FL 33802

Table 5. Effect of P rate on dry weight of perennial ryegrass tissue as affected by P source, ryegrass study.

P Rate (kg ha <sup>-1</sup> )	Dry weight (g)		
	P source		
	Turfite <sup>†</sup>	TKO	TSP
	Harvest 1, Run 1		
0	0.15	0.15	0.15
13	0.18	0.22	0.15
26	0.20	0.20	0.18
40	0.13	0.12	0.21
52	0.13	0.18	0.21
Regression	Q <sup>‡</sup>	NS	L
	Harvest 2, Run 1		
0	0.12	0.12	0.12
13	0.18	0.19	0.17
26	0.19	0.22	0.23
40	0.35	0.23	0.20
52	0.30	0.24	0.16
Regression	L	L	Q
	Run 2		
0	0.99	0.99	0.99
13	1.32	1.15	0.98
26	1.93	1.19	1.08
40	1.94	1.23	1.11
52	1.98	1.38	1.16
Regression	L	L	L

<sup>†</sup> Turfite: phosphite contained fertilizer; TKO: phosphite contained fertilizer; TSP: phosphate fertilizer

<sup>‡</sup> From linear regression, L= significant linear response within each P source and Harvest/Run; Q= significant quadratic response; NS = no linear or quadratic response.

Table 6. Effect of P rate on P content in perennial ryegrass tissue as affected by P source.

P Rate (kg ha <sup>-1</sup> )	Tissue P (mg kg <sup>-1</sup> )		
	P source		
	Turfite <sup>†</sup>	TKO	TSP
	Harvest 1, Run 1		
0	2,061	2,061	2,061
13	6,644	6,046	6,057
26	8,891	8,227	9,443
40	11,314	8,208	9,844
52	11,863	10,925	11,842
Regression	L <sup>‡</sup>	L	L
	Harvest 2, Run 1		
0	1,703	1,703	1,703
13	6,027	4,373	4,555
26	8,861	6,451	5,854
40	8,731	7,236	7,664
52	11,253	9,214	11,010
Regression	L	L	L
	Run 2		
0	3,700	3,700	3,700
13	5,700	5,760	5,860
26	5,980	6,440	7,040
40	7,520	7,600	7,960
52	8,320	9,100	8,920
Regression	L	L	L

<sup>†</sup> Turfite: phosphite contained fertilizer; TKO: phosphite contained fertilizer; TSP: phosphate fertilizer

<sup>‡</sup> From linear regression, L= significant linear response within each P source and Harvest/Run.

Table 7. Analysis of variance for bentgrass growth variables as affected by P rate, phosphite source and their interaction.

	P > F			
	Variable			
	Tissue dry weight	P content	P uptake	Root dry weight
	Run 1			
P Rate	0.1820	0.0037	0.0162	0.0890
P Source	< 0.0001	< 0.0001	< 0.0001	0.0010
Rate × Source	0.2996	0.0003	0.0005	0.0460
	Run 2			
P Rate	0.5440	< 0.0001	< 0.0001	0.4180
P source	< 0.0001	< 0.0001	< 0.0001	0.0190
Rate × source	0.3380	0.0046	0.4290	< 0.0001

Table 8. Effect of soil P rate on P content in bentgrass tissue as affected by P source.

P Rate (kg ha <sup>-1</sup> )	Tissue P (mg kg <sup>-1</sup> )		
	P source		
	None	Title Phyte	Alude
	Run 1		
0	2,464 c	4,306 b	5,473 a
15	3,199 c	4,075 b	5,043 a
30	2,992 b	4,299 a	4,853 a
60	4,082 b	4,848 a	4,870 a
120	4,264 b	4,356 a	5,042 a
Regression	Q <sup>†</sup>	NS	Q
	Run 2		
0	2,820 c	7,680 b	9,340 a
15	3,860 c	8,160 b	9,380 a
30	4,600 b	8,740 a	9,120 a
60	4,860 b	8,500 a	10,000 a
120	6,680 b	10,620 a	9,880 a
Regression	L	Q	NS

<sup>†</sup> From linear regression, L= significant linear response within each P source and Harvest/Run; Q= significant quadratic response; NS = no significant linear or quadratic response.

The difference of tissue P means between sources were analyzed at each P rate. Tissue P means followed by the same letter are not significantly different at the  $\alpha= 0.05$  level as determined by the Tukey test.

Table 9. CaCl<sub>2</sub> extractable P as affected by P rate and P source for incubation study, sampled at September.

P Rate (kg ha <sup>-1</sup> )	CaCl <sub>2</sub> extractable P			
	mg kg <sup>-1</sup>			
	TKO	Turfite	Title Phyte	TSP <sup>†</sup>
	September			
0	19.9	19.9	19.9	19.9
56	16.7	21.5	22.5	10.6
84	22.7	35.9	14.9	16.8
112	17.1	8.8	12.4	25.0
140	12.0	14.6	24.0	12.0
Regression	NS	NS	NS	NS

<sup>†</sup> Triple super phosphate

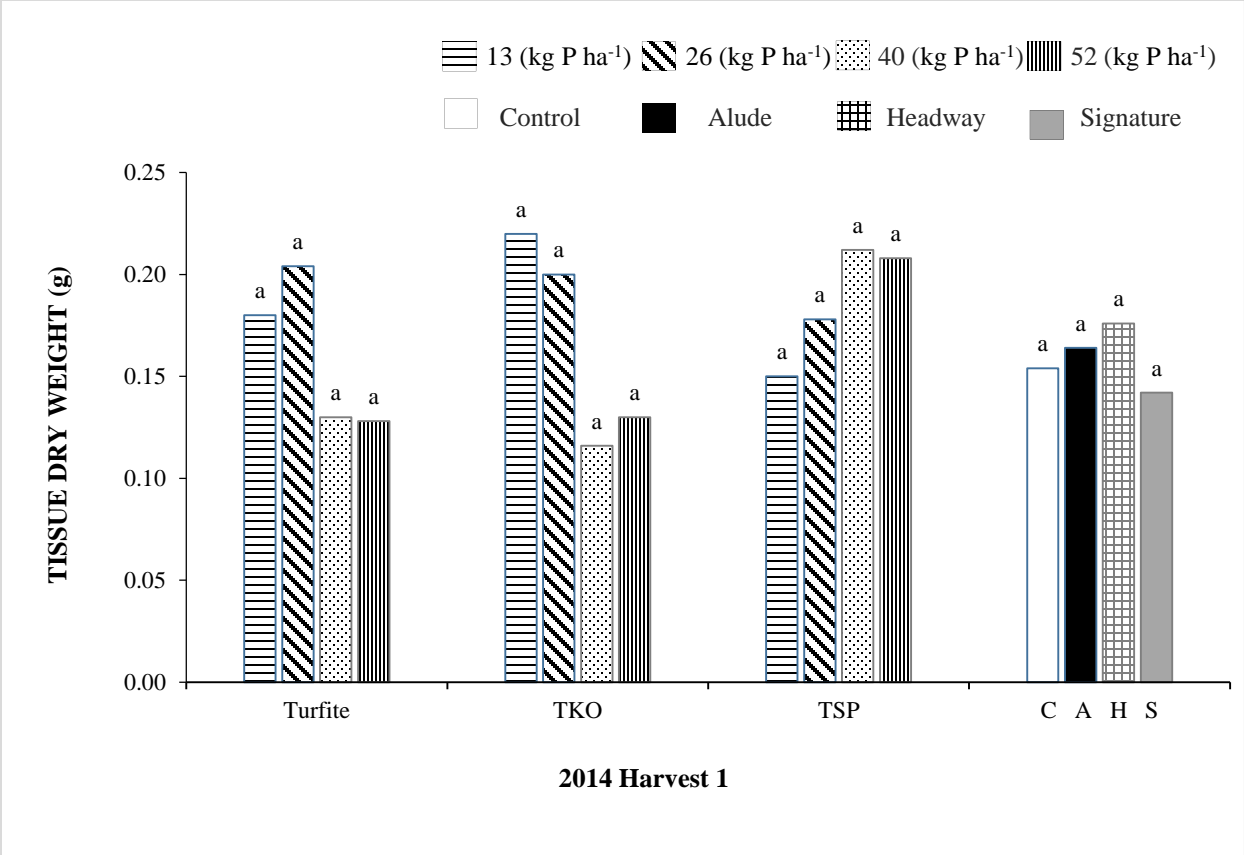


Fig 1. Effect of increasing rates of P rate and P additive on perennial ryegrass tissue dry weight, Run 1, Harvest 1. Statistics shown were performed on individual means at the  $\alpha = 0.05$  level using the Tukey test.

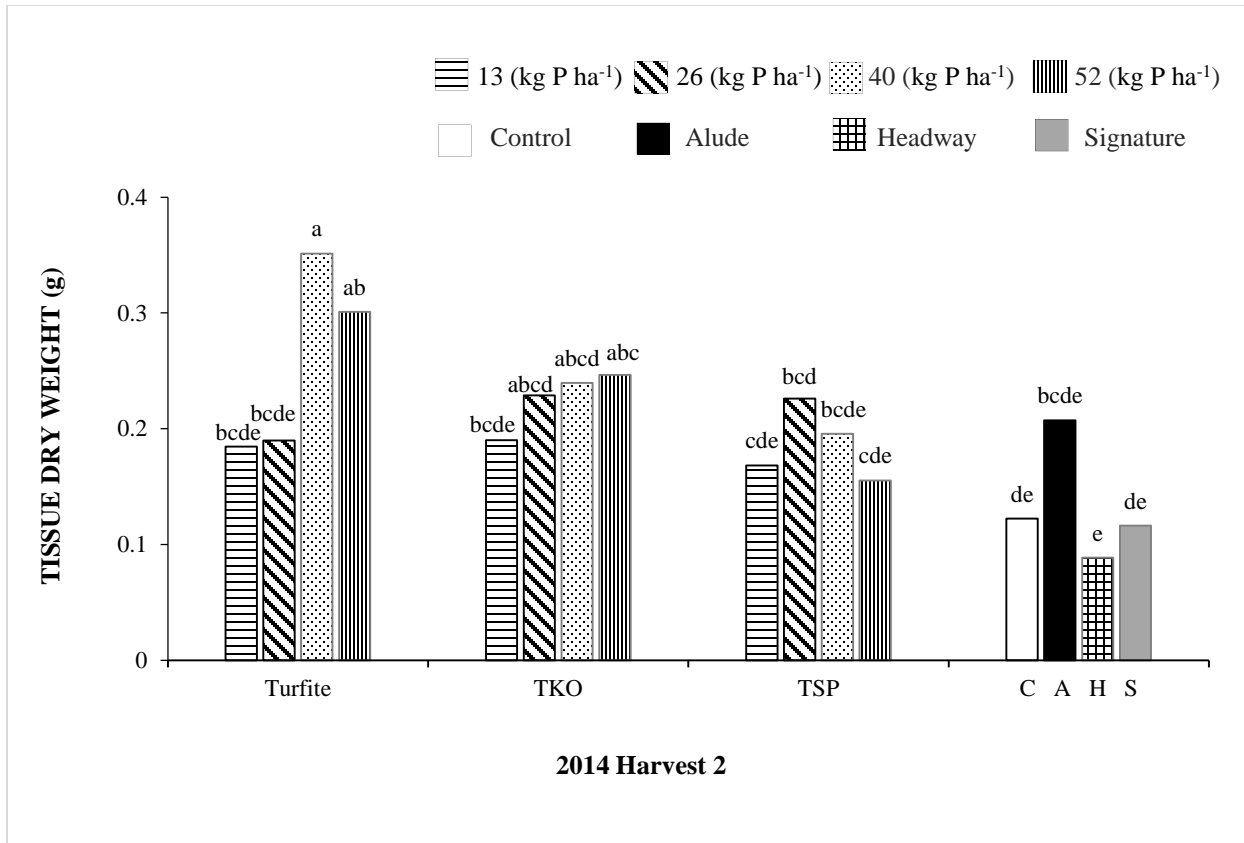


Fig 2. The effect of increasing rates of P rate and P additive on perennial ryegrass tissue dry weight for Run 1, Harvest 2. Statistics shown were performed on individual means at the  $\alpha = 0.05$  level using the Tukey test.



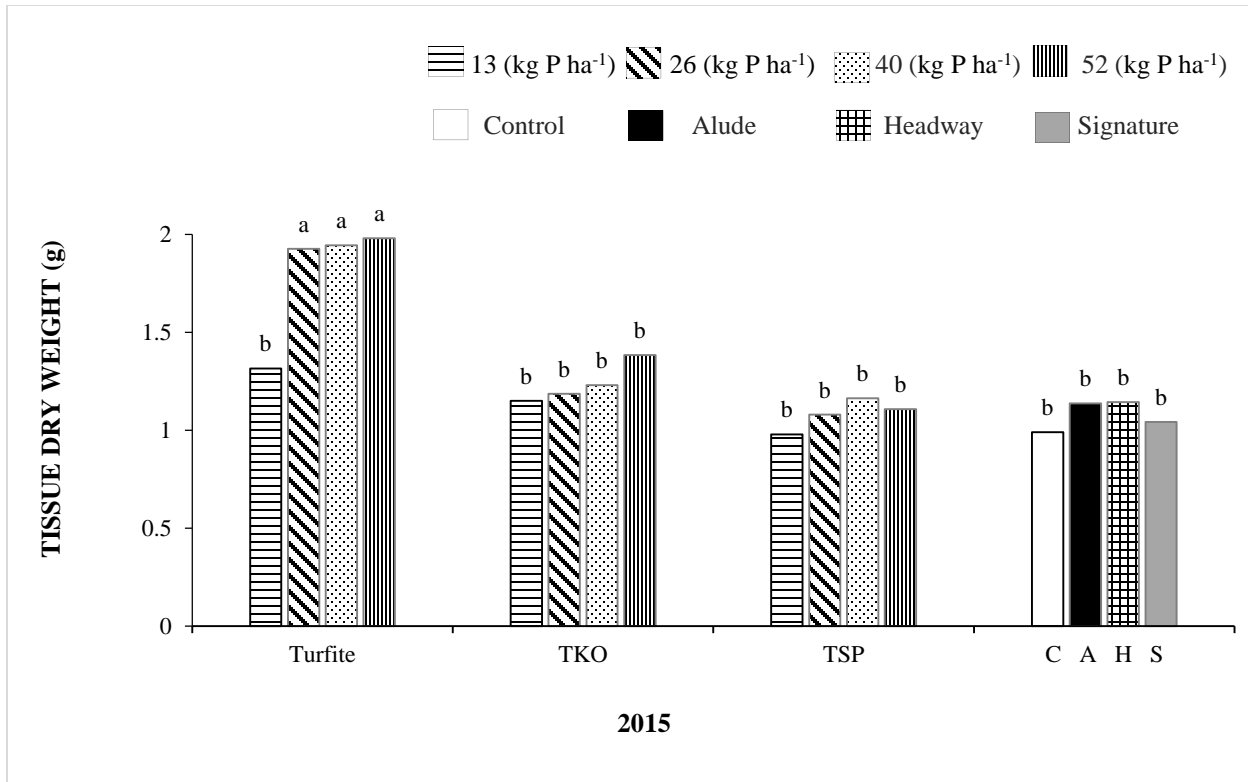


Fig 3. Effect of increasing rates of P rate and P additive on ryegrass tissue dry weight, Run 2. Statistics were analyzed cross all treatments. Ryegrass tissue dry weight means followed by the same letter are not significantly different at the  $\alpha = 0.05$  level as determined by the Tukey test.

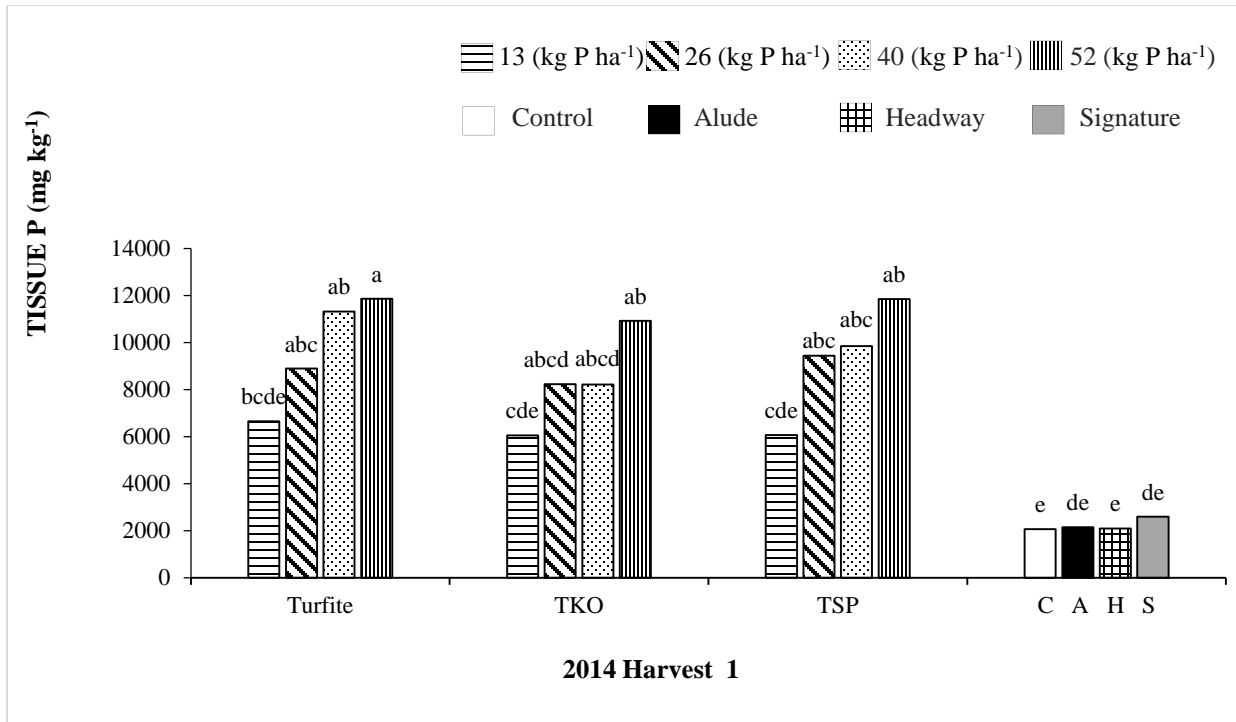


Fig 4. Ryegrass tissue P concentration as affected by P rate, P source and fungicide, Harvest 1 of Run 1. Tissue P means followed by the same letter are not significantly different at the  $\alpha = 0.05$  level as determined by the Tukey test.

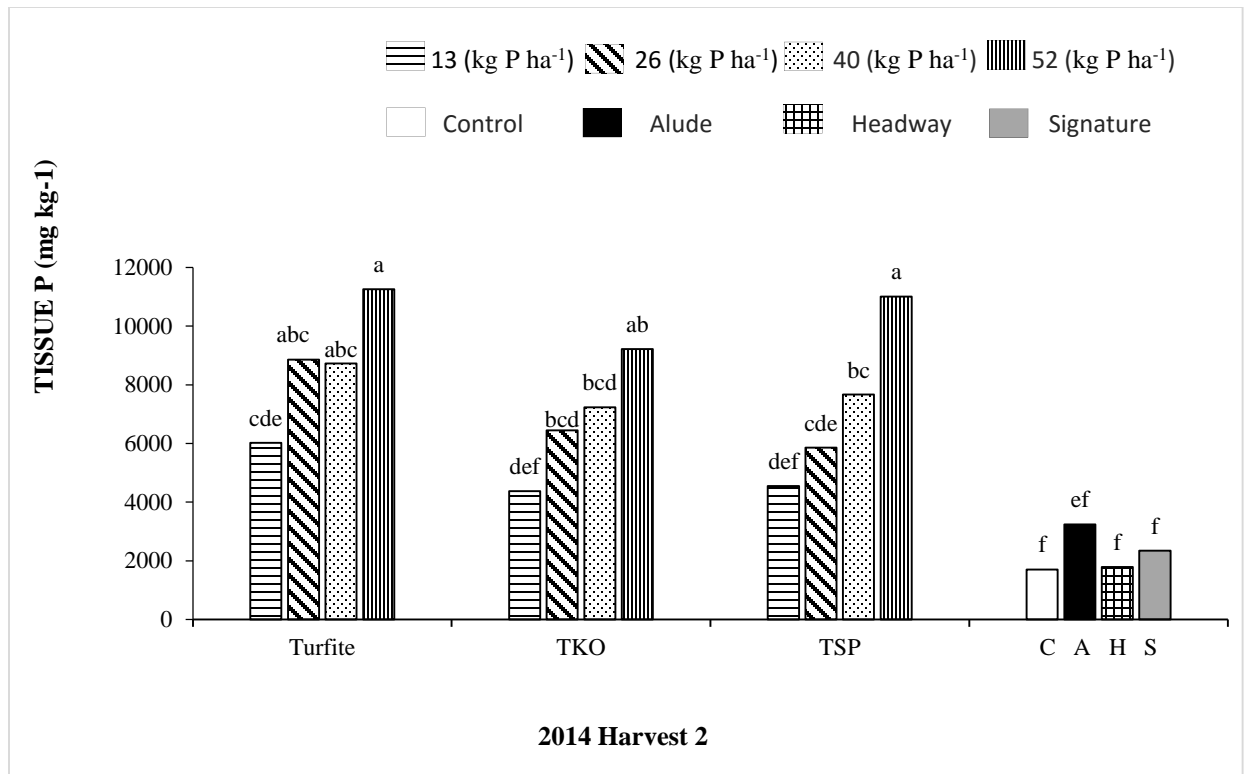


Fig 5. Ryegrass tissue P concentration as affected by P rate, P source and fungicide, Harvest 2 of Run 1. Tissue P means followed by the same letter are not significantly different at the  $\alpha = 0.05$  level as determined by the Tukey test.

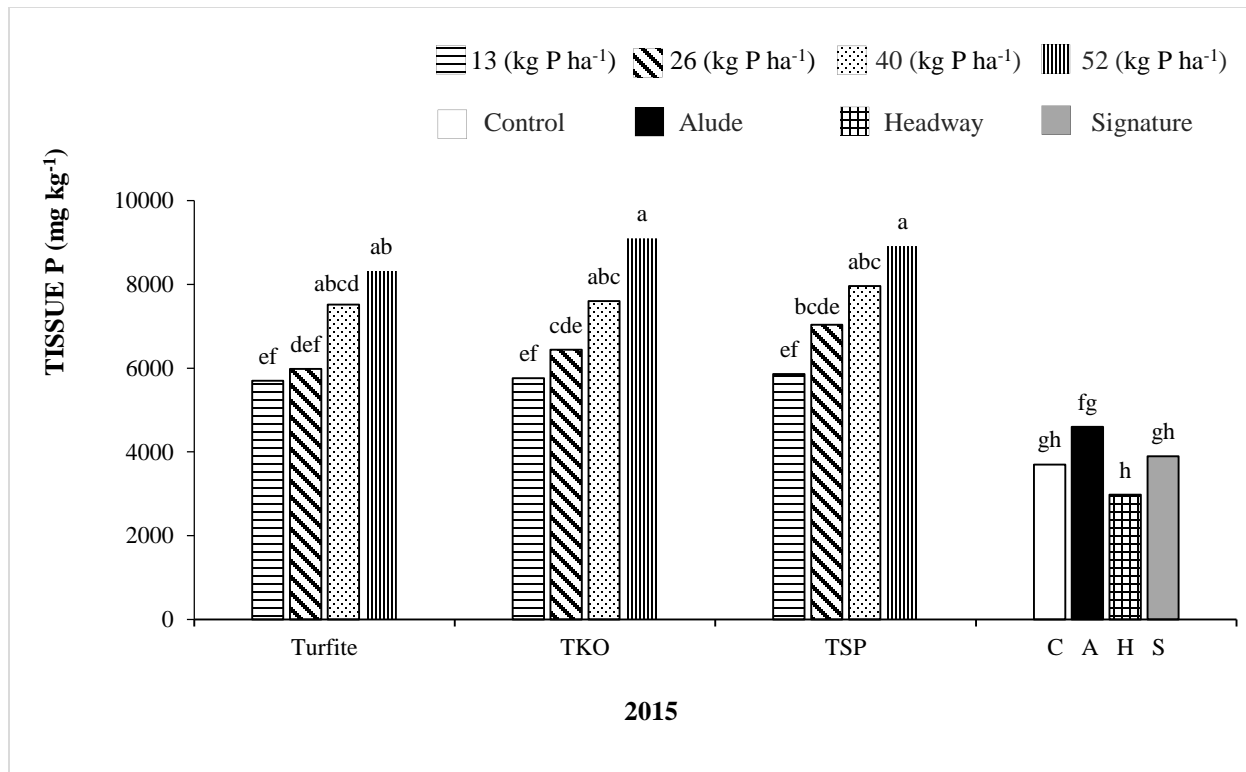


Fig 6. Ryegrass tissue P concentration as affected by P rate, P source and fungicide, Run 2. Tissue P means followed by the same letter are not significantly different at the  $\alpha = 0.05$  level as determined by the Tukey test.

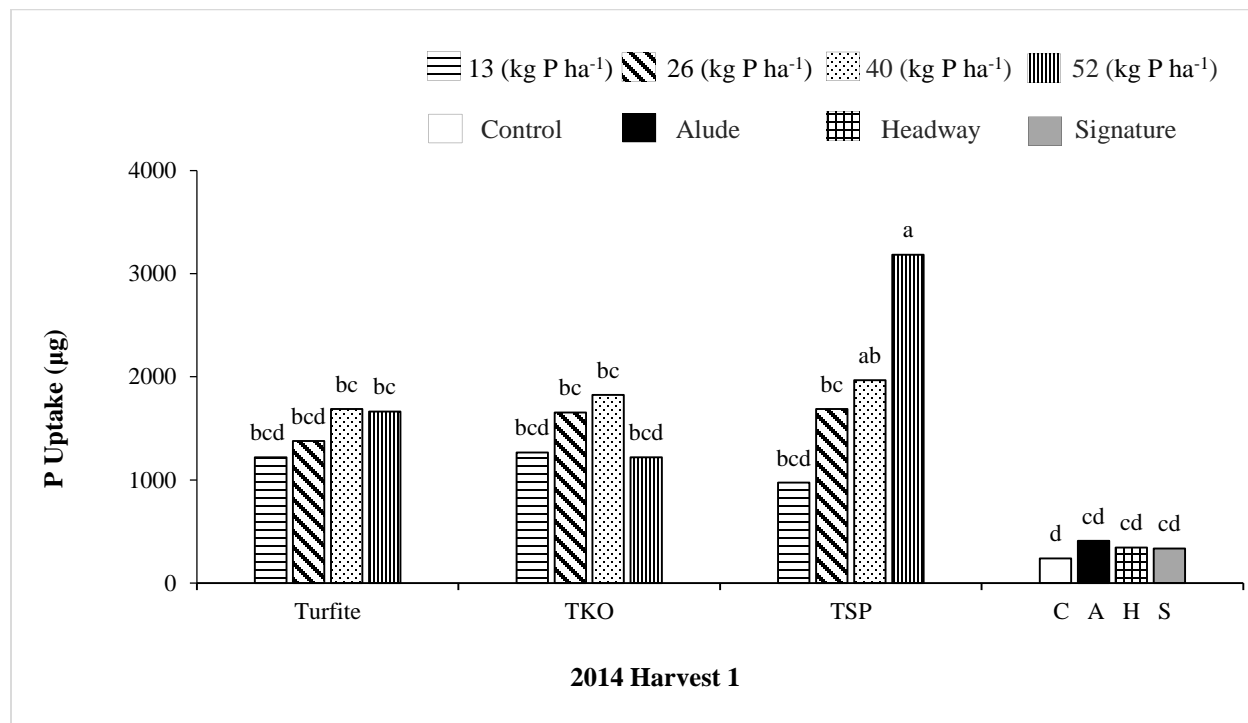


Fig 7. Uptake of P by perennial ryegrass as affected by P rate, P source and fungicide, Harvest 1, Run 1. Acid extractable tissue P means followed by the same letter are not significantly different at the  $\alpha=0.05$  level as determined by the Tukey test.

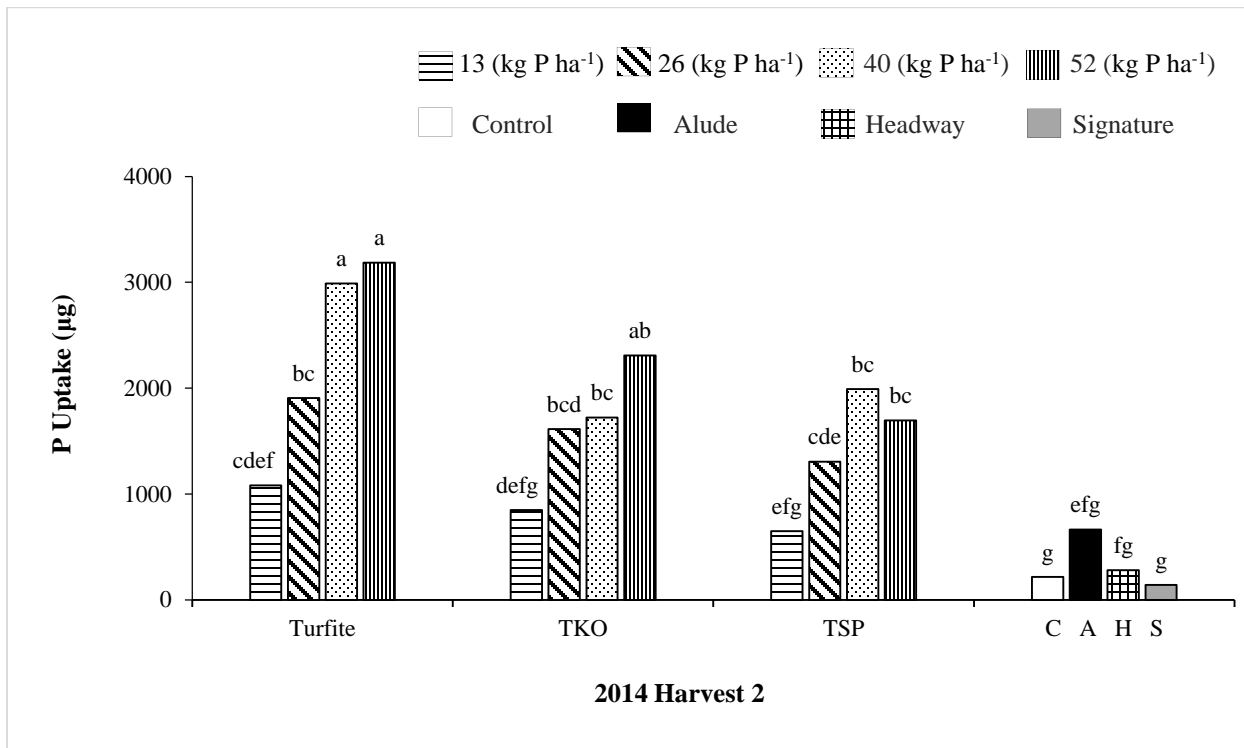


Fig 8. Uptake of P by perennial ryegrass as affected by P rate, P source and fungicide, Harvest 2, Run 1. Acid extractable tissue P means followed by the same letter are not significantly different at the  $\alpha = 0.05$  level as determined by the Tukey test.

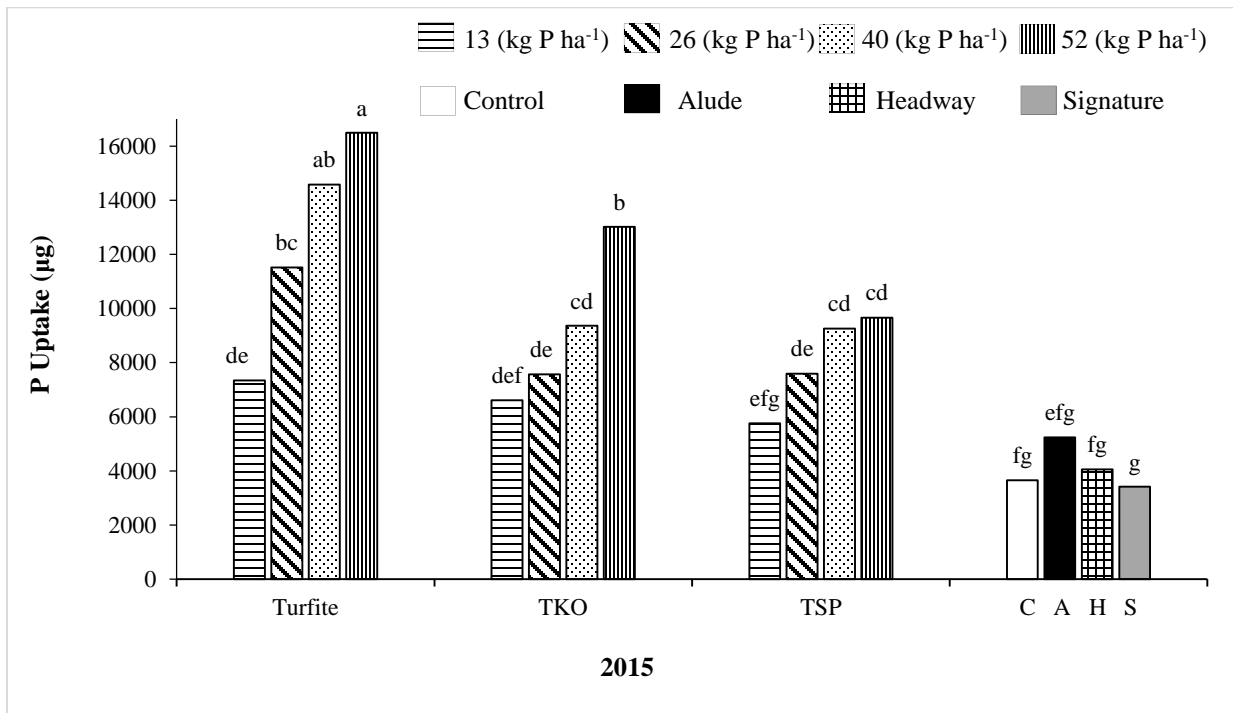


Fig 9. Uptake of P by perennial ryegrass as affected by P rate, P source and fungicide, Run 2. Acid extractable tissue P means followed by the same letter are not significantly different at the  $\alpha = 0.05$  level as determined by the Tukey test.

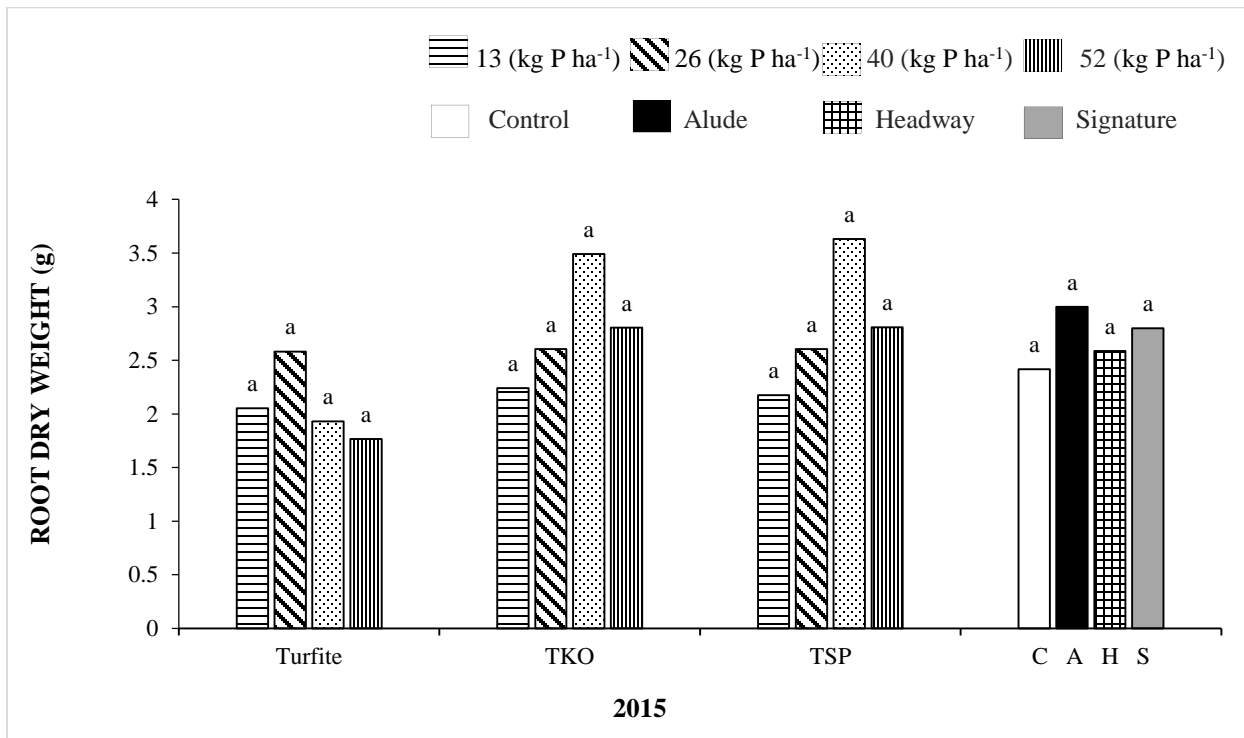


Fig 10. Dry root weight of perennial ryegrass as affected by P rate, P source and fungicide, Run 2. Treatments with the same letter above the bars are not significantly different ( $\alpha = 0.05$ ) within an experiment.



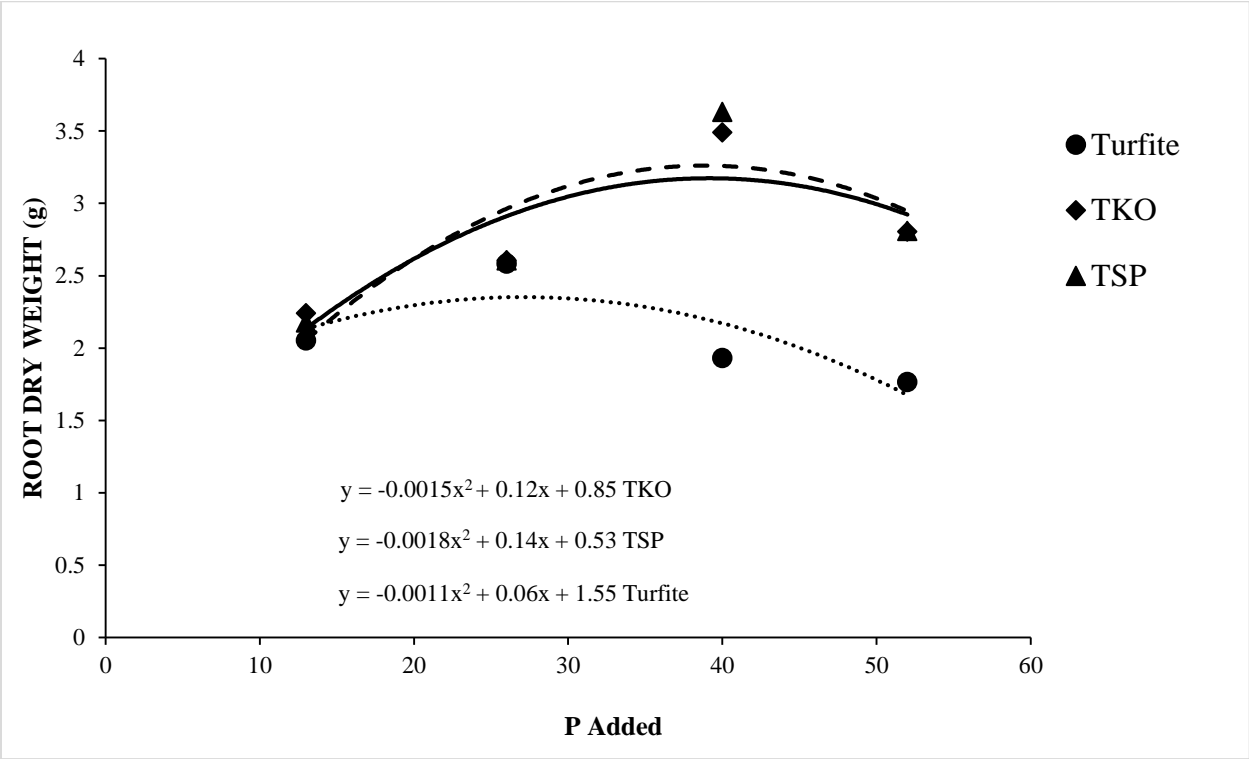


Fig 11. Dry weight of perennial ryegrass roots as affected by P rate and P source. Run 2 only. Turfite, TKO are phosphite contained fertilizers and TSP are phosphate contained fertilizer.

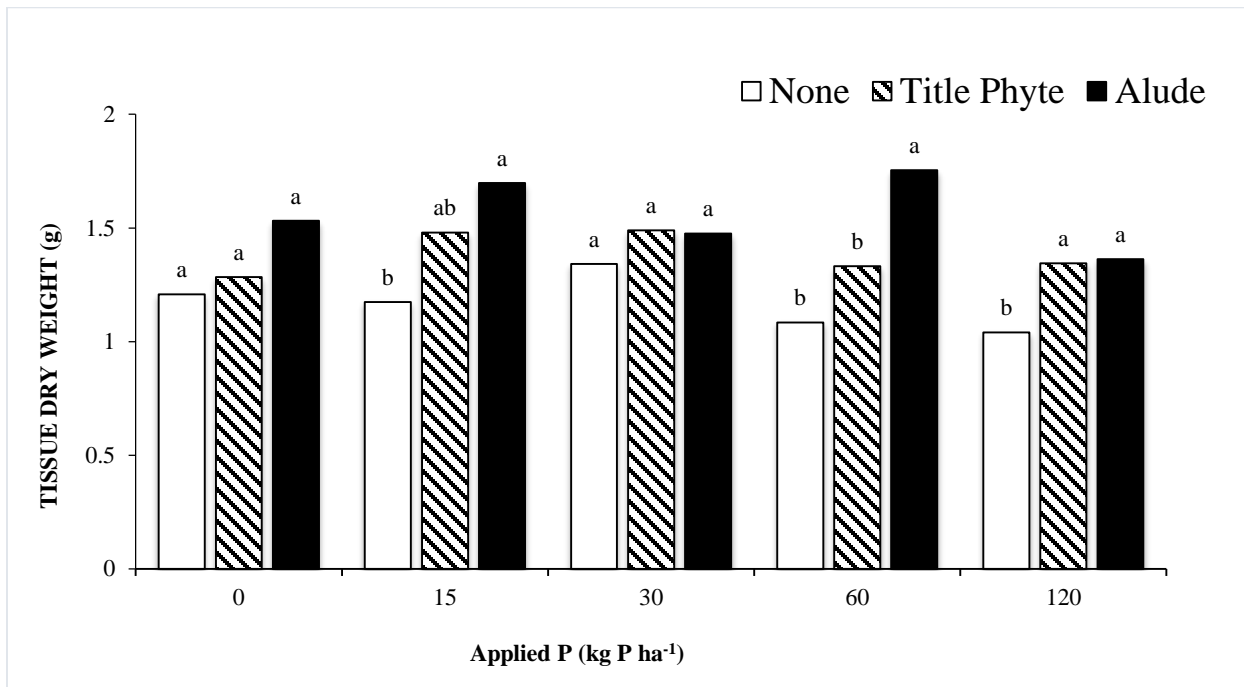


Fig 12. Effect of P fertilizer (applied as triple super phosphate) and phosphite products on tissue dry weight of creeping bentgrass, Run 1. Treatments with the same letter above the bars are not significantly different ( $\alpha = 0.05$ ) within an experiment.

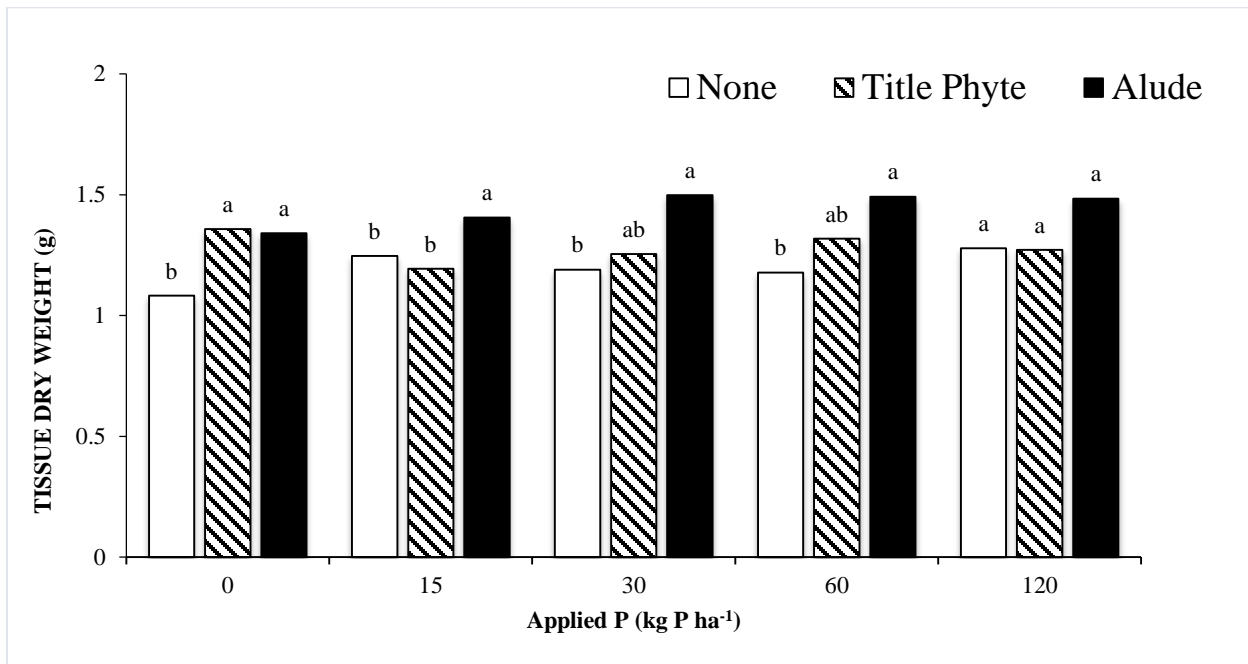


Fig 13. Effect of P fertilizer (applied as triple super phosphate) and phosphite products on tissue dry weight of creeping bentgrass, Run 2. Treatments with the same letter above the bars are not significantly different ( $\alpha = 0.05$ ) within an experiment.

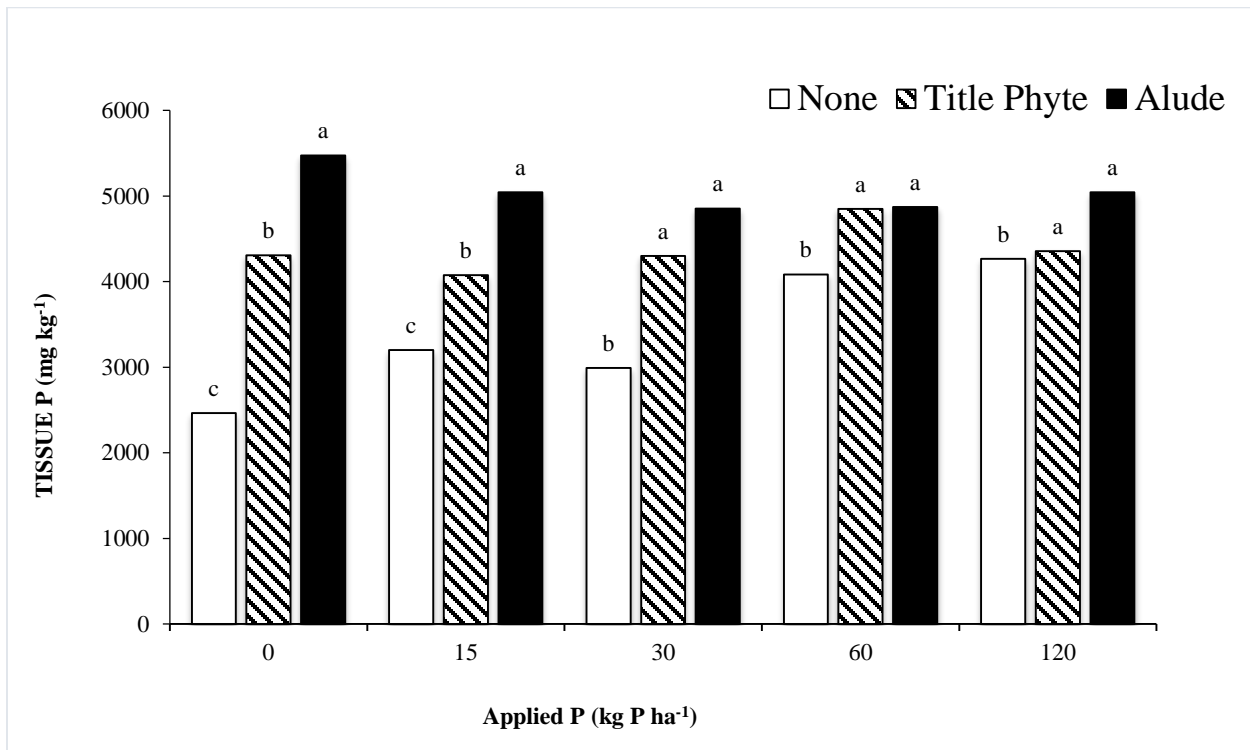


Fig 14. Effect of P rate (as TSP) and phosphite products on tissue P in creeping bentgrass, Run 1. Treatments with the same letter above the bars are not significantly different ( $\alpha = 0.05$ ) within an experiment.

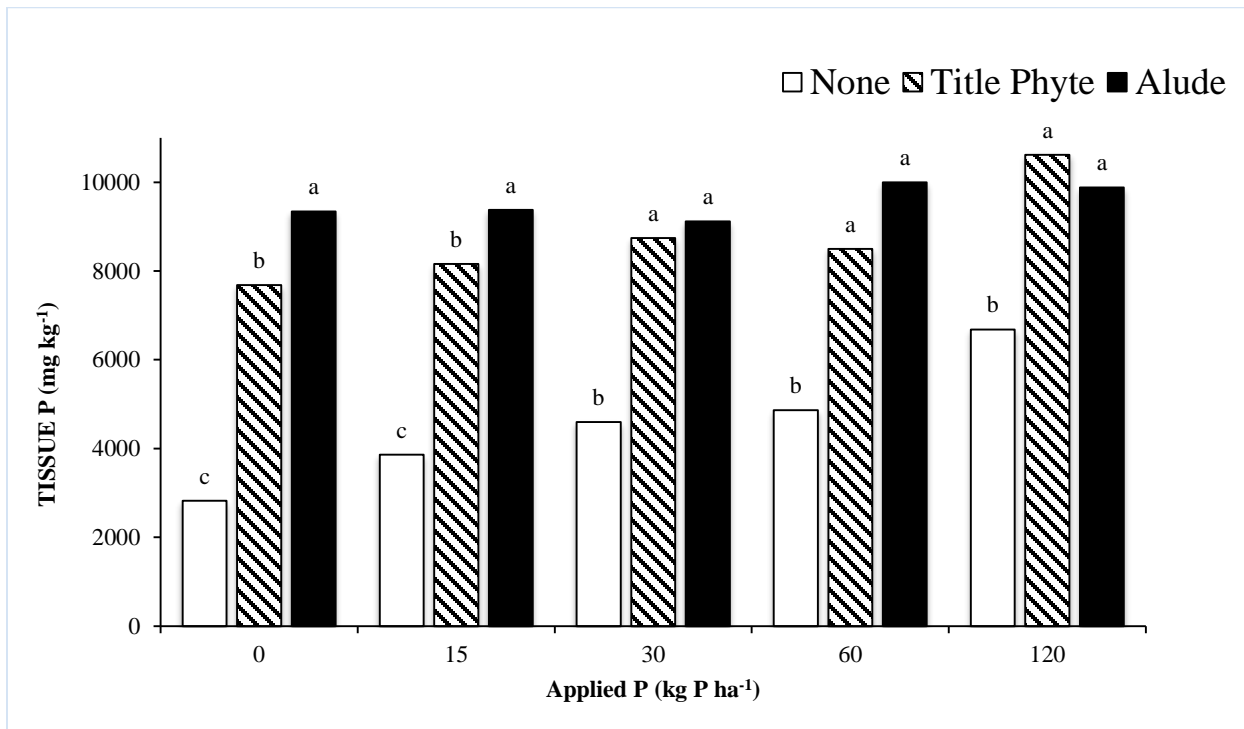


Fig 15. Effect of P rate (as TSP) and phosphite products on tissue P in creeping bentgrass, Run 2. Treatments with the same letter above the bars are not significantly different ( $\alpha = 0.05$ ) within an experiment.

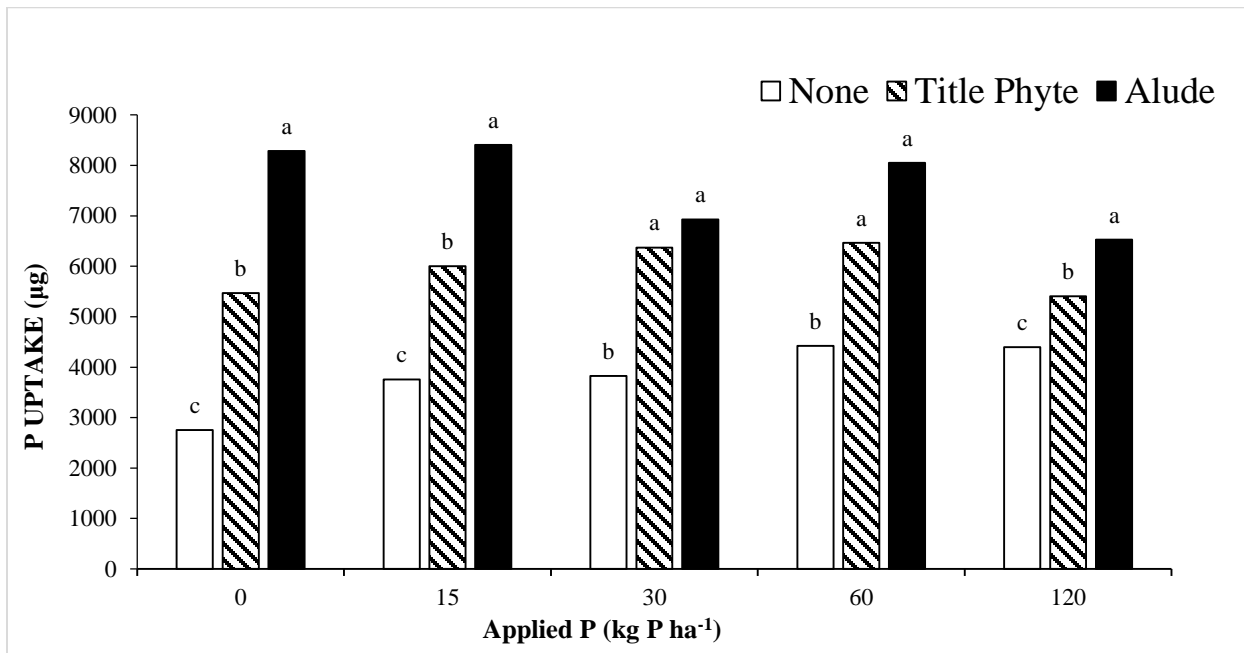


Fig 16. Effect of P rate (as TSP) and phosphite products on P uptake by creeping bentgrass, Run 1. Treatments with the same letter above the bars are not significantly different ( $\alpha = 0.05$ ) within an experiment.

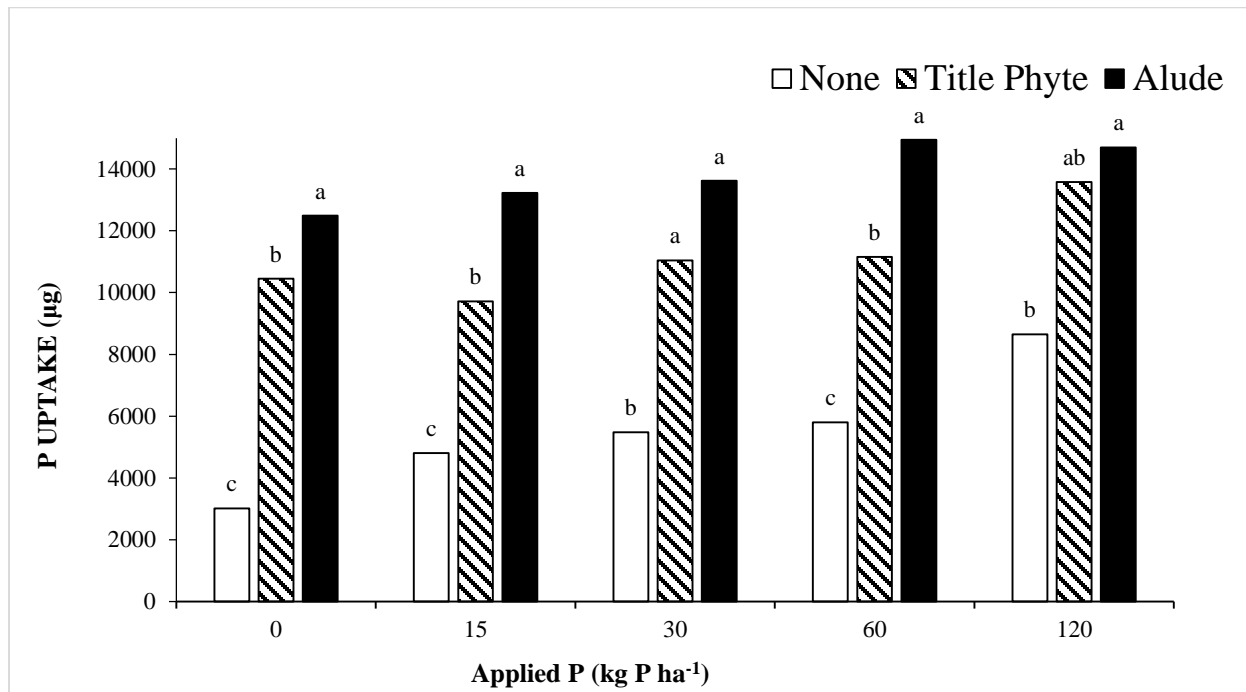


Fig 17. Effect of P rate (as TSP) and phosphite products on P uptake by creeping bentgrass, Run 2. Treatments with the same letter above the bars are not significantly different ( $\alpha = 0.05$ ) within an experiment.

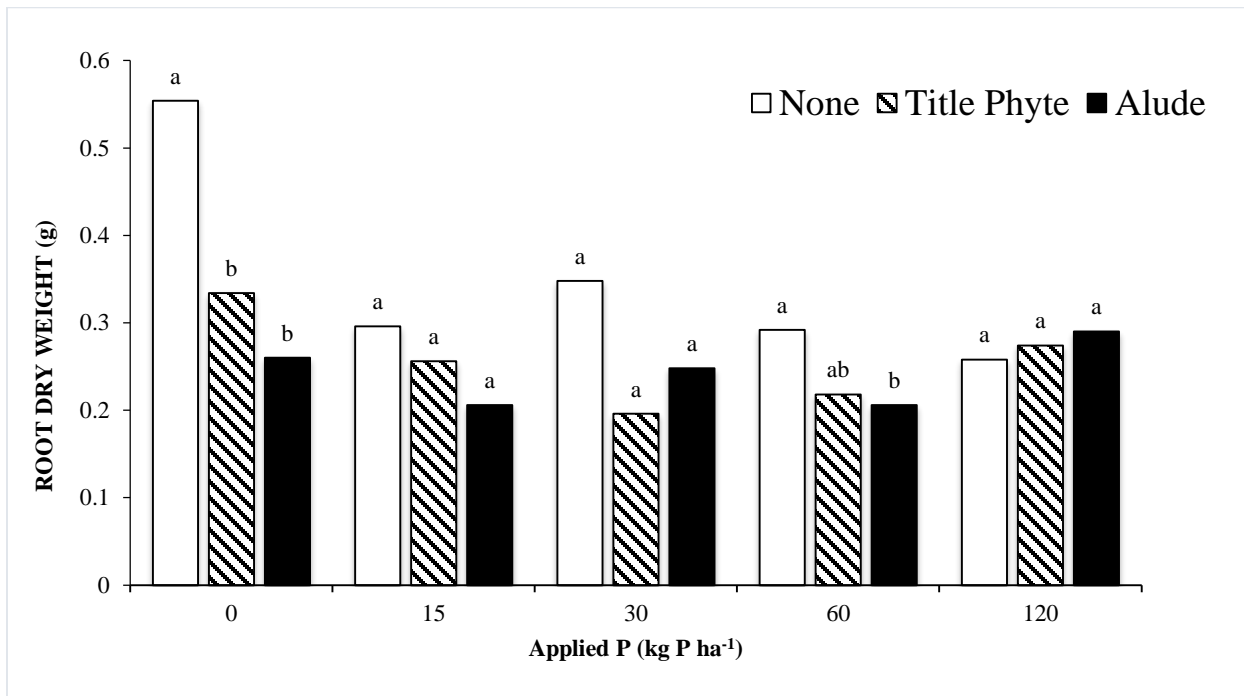


Fig 18. Effect of P rate (as TSP) and phosphite products on creeping bentgrass root dry weight, Run 1. Treatments with the same letter above the bars are not significantly different ( $\alpha = 0.05$ ) within an experiment.



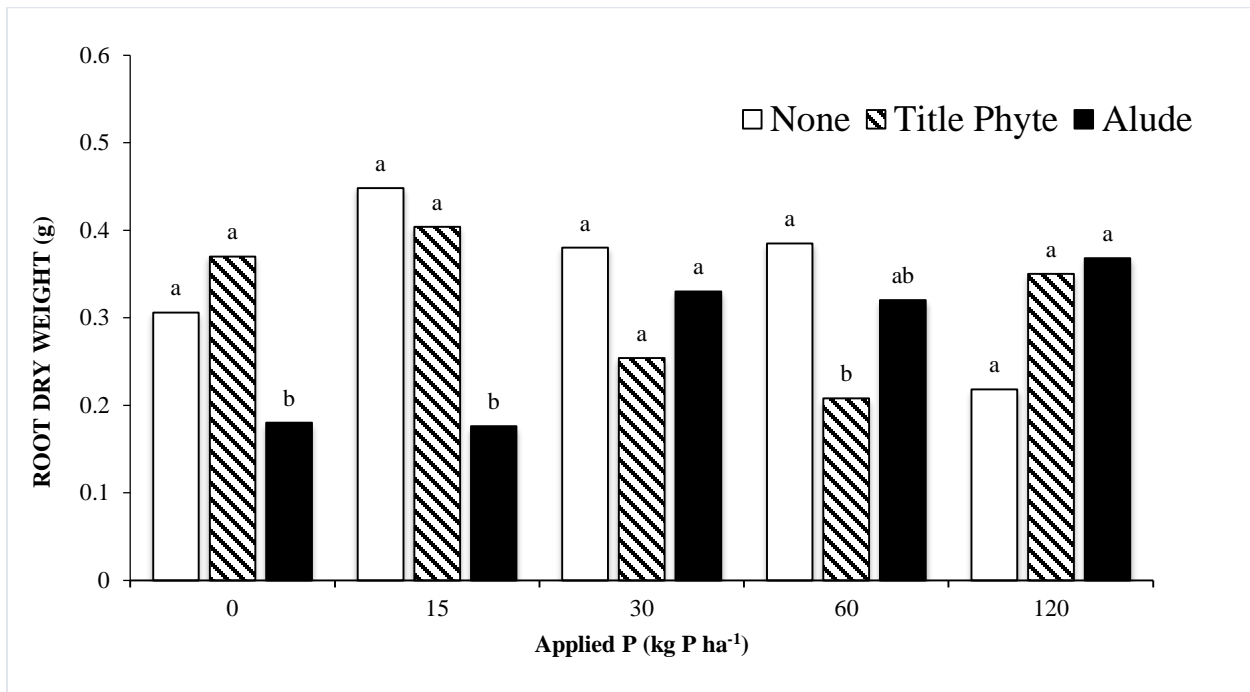


Fig 19. Effect of P rate (as TSP) and phosphite products on creeping bentgrass root dry weight, Run 2. Treatments with the same letter above the bars are not significantly different ( $\alpha = 0.05$ ) within an experiment.

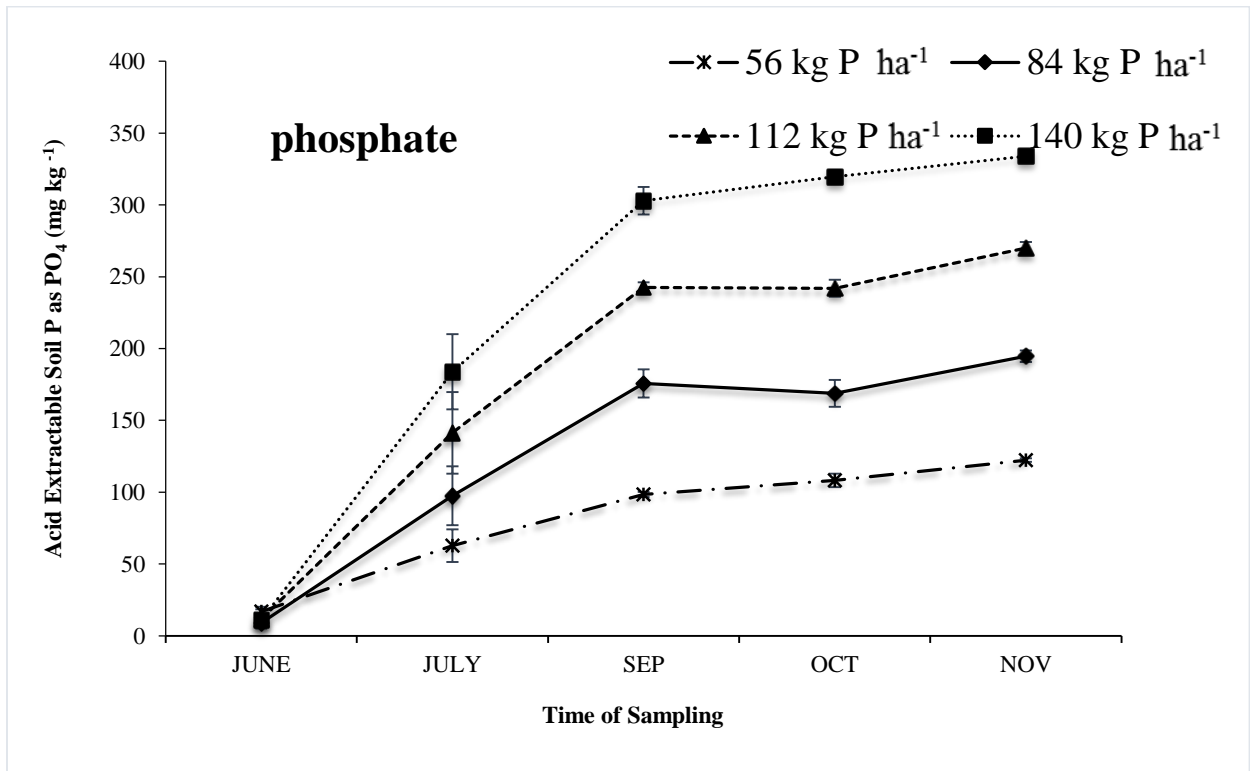
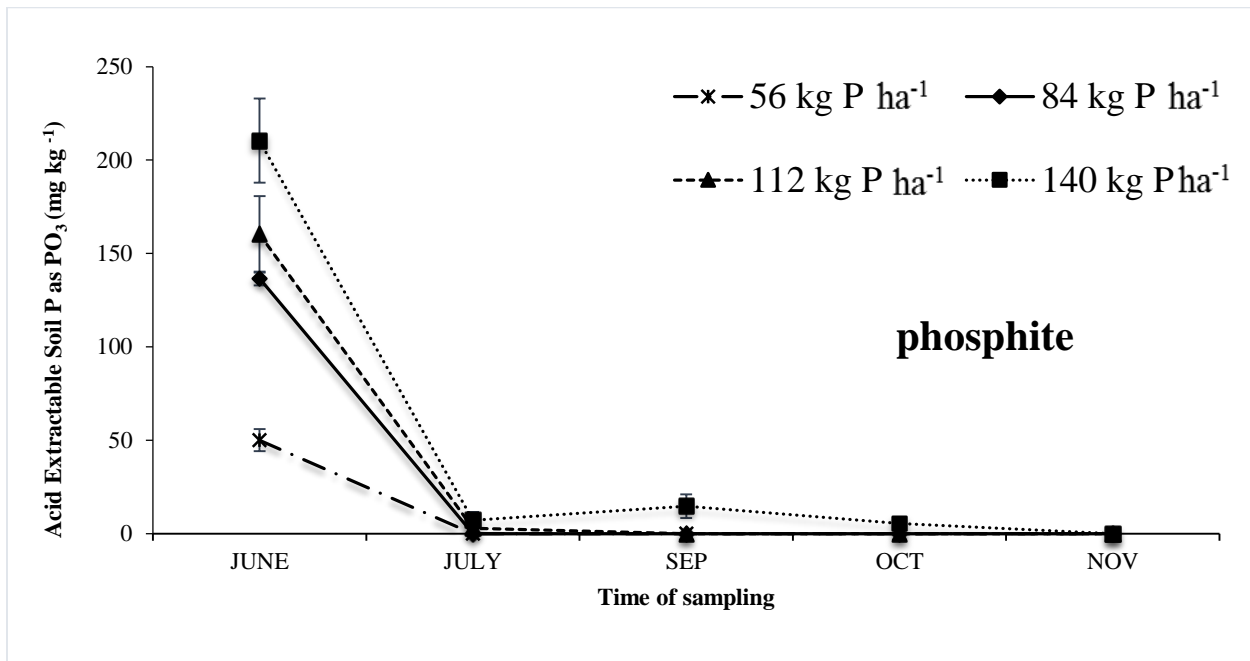


Fig 20. Effect of increasing rates of P supplied as phosphite (TKO) and sampling month on Mehlich-1 extractable soil P as phosphite (top) and phosphate (bottom) in Marvyn loamy sand. Error bars represent the standard error of the mean (SEM).

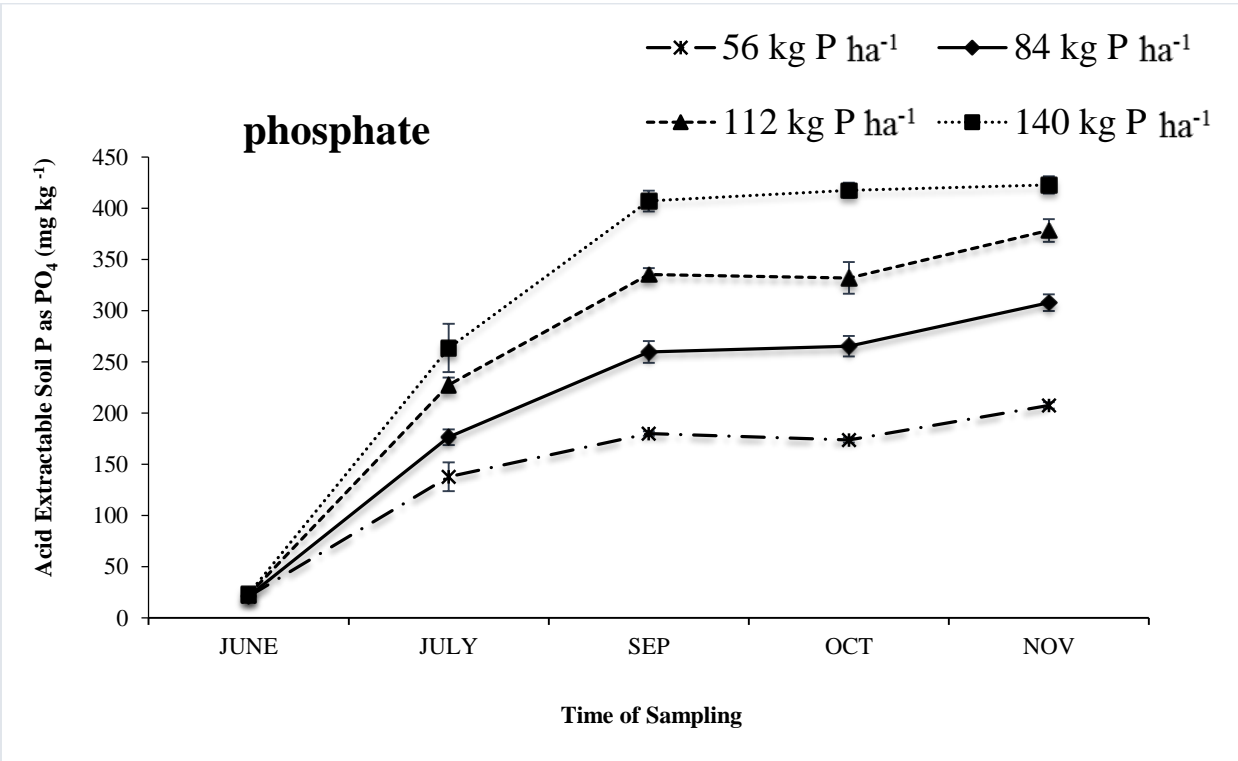
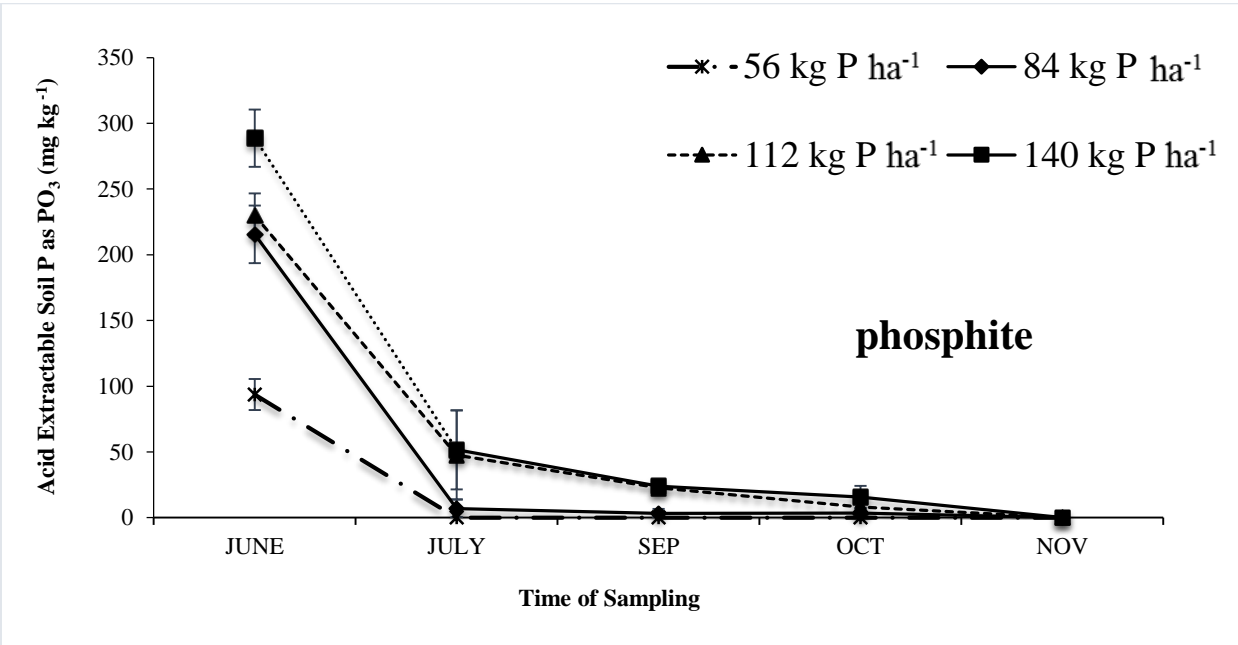


Fig 21. Effect of increasing rates of Title Phyte and sampling month on Mehlich-1 extractable soil P as phosphite (top) and phosphate (bottom) in Marvyn loamy sand. Error bars represent the standard error of the mean (SEM).

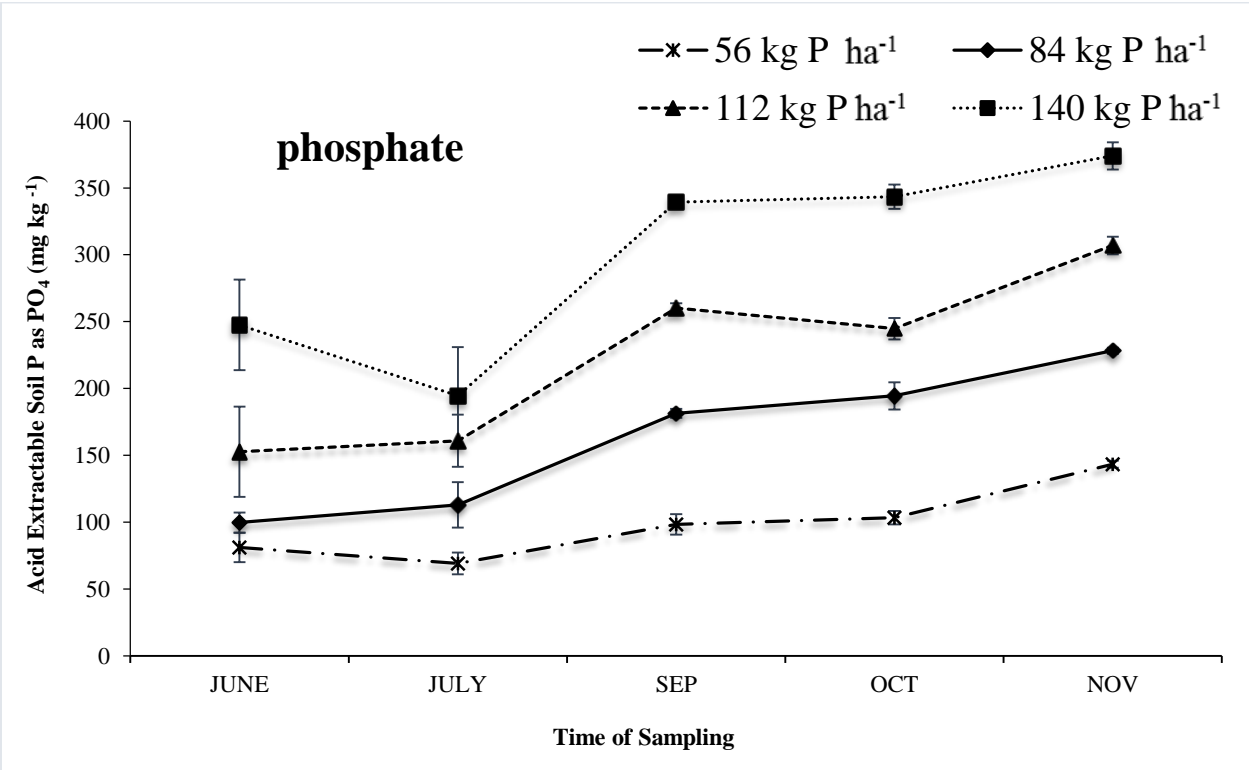
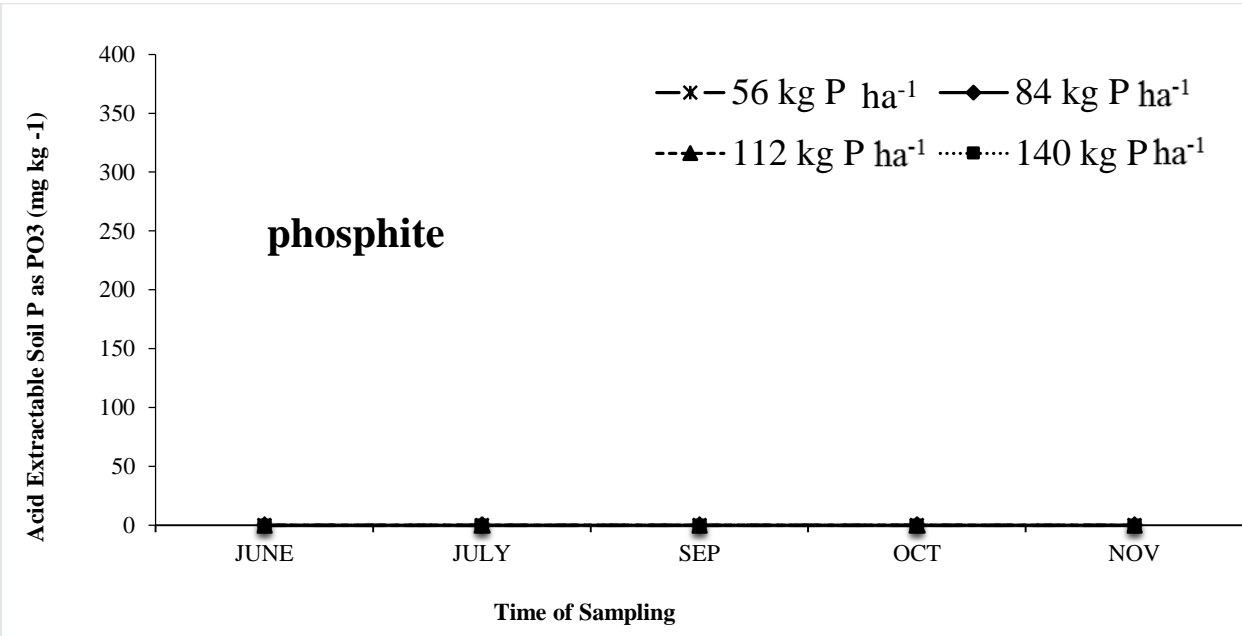


Fig 22. Effect of increasing rates of Triple super phosphate and sampling month on Mehlich-1 extractable soil P as phosphite (top) and phosphate (bottom) in Marvyn loamy sand. Error bars represent the standard error of the mean (SEM).

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